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PEARL FISHERIES OF SULU

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SEVEN PLATES

INTRODUCTION

January 3, 1930, I left Manila for Sulu for the purpose of investigating the present conditions surrounding the pearling industry of the Sulu Archipelago. One month was spent in the study, and my investigations were conducted chiefly in the vicinities of Zamboanga, Basilan, Jolo, Siasi, Bongao, and Sitanki. The most important men who were familiar or intimately connected with the industry, such persons as Government officials, Chinese, Japanese, and Moro pearl merchants, divers, and fishermen, of the places visited, were consulted or interviewed. The information obtained from one locality was checked up in another. In the following paragraphs is embodied all that I could learn, through information and personal observation, about the present situation of the pearl fishery of the Sulu Archipelago.

The Sulu Archipelago is an extensive natural pearling ground. It is located between 4° and 7° north latitude and is bounded by the Sulu Sea on the north and the Celebes Sea on the south and east. The archipelago extends for its entire length of 137 miles in a southwesterly direction from Basilan to Borneo. It consists of two main groups of islands—the Jolo group and the Tawitawi group—which are again divided into thirteen small groups with about one hundred thirty fairly large islands and some one hundred seventy islets and reefs. The islands and

The Pangutarang pearl beds.—These beds are considered one of the richest pearling grounds in Sulu. The banks east of Basbas Island are particularly productive. January 30 last, the *Kotohira* brought to Jolo five hundred twenty-nine shells fished in the vicinity of Basbas, Cumilan, and Usada Islands. The Moro diver informed me that the north wind had been too strong for the boat to operate around the adjacent beds, which are known to be rich in shells. It was said that the *Kotohira* on this trip obtained one pearl valued at 800 pesos; I went to see the Chinese manager of the vessel about this but he was noncommittal. It is this secretive attitude of the pearl merchants that makes any estimate of the income from the sales of pearls taken in Sulu waters rather uncertain and unreliable. The Pangutarang beds are in no danger of exhaustion.

The Jolo pearl beds.—The productive sections of these beds are the shoals and channels between the islands north of Jolo and the channel in front of the town of Jolo. The Moros for over a hundred years have been diving for pearls in this region, and there has been no apparent diminution in the yield of pearl shells. The beds are just as rich as ever. However, it is believed that the conditions in this locality are not favorable for the production of pearls. Rarely, if ever, are pearls found in the shells from the Jolo beds.

January 18 I was a guest on board the pearler *Aki*. This is a motor boat equipped with a modern diving outfit. Hadji Gulamu Rasul and Mr. Kashiwagi, president of the Sulu Pearling Co., Inc., arranged the trip. The boat worked in three different places in the channel in front of the town of Jolo. Two Japanese divers made the three dives of twenty-five minutes each in depths of from 22 to 25 fathoms. Some of the shells secured were already old, broken, or encrusted. There were six good shells of large size. The divers informed me that they found a goodly number of young shells of various sizes on the bottom, which is of fine ooze.

January 31 last, I boarded the *Sumiyoshi*, another motor yacht with motor pump, for the purpose of examining the beds in the vicinity of Marungas Island. The weather was fair, but the captain, a Visayan-Moro, and the diver, a Capiceño, and the Japanese supercargo, all stated that it would be inexpedient to go farther north; accordingly, the operation was confined to the southern waters of Marungas at a depth of 24 fathoms. Two dives of thirty minutes each were made. Twelve shells were ob-

tained, six of which were good and large, four large but broken and incrusted, and two undersized. The good shells were opened, but no pearls were found. Moro naked divers on vintas were working around Taglibi, Mainbung, and Parang. It appears that the Jolo beds are in no danger of depletion.

The Laparan pearl beds.—The many small islands, islets, and reefs that comprise these beds, lying well northward in the Sulu Sea, have favorable pearl-shell patches, and it has been reported that the natural supply could withstand without damage the operation of the present pearling fleet. A rich area has been recently discovered near Cap Island. In certain deep places pearl oysters are plentiful, but they are generally grouped too closely and the majority of them are old and worm eaten, perforated by boring sponges, or damaged by other natural enemies. Working these beds regularly would remedy this condition, and would in all probability increase the yield and improve the grade of shells. Some excellent pearls have been obtained from these grounds.

The Tapul pearl beds.—The pearl-shell patches of this area are more or less localized in the channels between Siasi and Lugus and Tapul. The Japanese and Moro divers who have worked on these beds for years informed me that there are plenty of shells here, but the water is deep and the current is usually so swift that operation is difficult. Excellent pearls have been secured from this region. In the shallow water around these islands Moro naked divers obtain plenty of shells, which are sold to the Chinese in Siasi.

The Tawitawi pearl beds.—These beds comprise the most prolific pearling grounds in the Sulu Archipelago and, as far as known, in the entire Philippines. Rich pearl-shell banks are located in the shallow water around Bubuan, Magpeos, Tagao, and South Ubian. In most of these places the water is too shallow for a pearling boat to operate. It is around this region that hundreds of Moro naked divers, the majority of whom are natives of South Ubian, find pleasure and profit in an occupation for which they have been trained. In the vicinity of Bongao good shells are found, but they generally occur singly. The deep sea around Cacataan Island abounds with shells of excellent grade, and pearls of the first water have been obtained from them. It is interesting to note that the majority of the shells taken from these beds have blisters, which invariably contain good pearls, especially when formed in the thicker portion of the

shell. The Tawitawi pearl beds are in good condition and could sustain the operation of a large pearling fleet without damage to the fisheries, which are constantly supplied by the local breeds as well as by those from the adjacent grounds.

The Sibutu pearl beds.—Nothing very definite is known about the pearl-shell patches in this area. Although the region as a whole has not been intelligently examined, apparently, it is rich in pearl shells, since the physical conditions surrounding the islands, reefs, and shoals west of Sibutu Passage are conducive to the growth of pearl oysters. According to Mr. Machlan, postmaster, custom collector, and deputy governor at Sitankai, the Moros occasionally bring into town some pearl shells secured from the shoals of Bulubulu, Tumindao, and Wooded Islands, but their harvest has been very small. In all probability in the deep water considerable numbers of shells could be found, but whether the Sibutu pearl beds are sufficiently extensive so that a large pearling fleet could find profitable work is doubtful. However, it was reported by the pearlers who had worked around the islands west of Alice Channel that pearl shells are plentiful there. This strengthens the probability that the Sibutu pearl beds are as rich as those located near the mouth of Darvel Bay, for the regions are contiguous and represent the connecting links between the Sulu Archipelago and Borneo.

REMARKS

In brief, as far as I could learn, these nine pearl beds are in no immediate danger of depletion. It is possible, however, that some rich pearl patches localized around certain islands may suffer from overfishing, for there are several such grounds that, because they are sheltered practically at all seasons, permit continuous operation of the pearlers and naked divers. However, this situation will eventually solve itself, because as soon as fishing on these grounds becomes unprofitable, the pearlers will naturally seek other places, leaving the beds to recuperate. The Sulu pearl banks usually recover in a comparatively short time. It has been observed that oyster shells in Sulu waters grow rather rapidly, there being no cold spells as in Japan and Australia to retard their growth activity. The Philippine pearl oyster (*Margaritifera maxima*) is sexually mature in two years and attains the "present legal size;" that is, 14 centimeters nacre measurement in about three or four years. As a matter of fact, divers do not and cannot take out all the available fishable (legal) oysters on a bank at a fishery. Thus, even under such

seemingly adverse circumstances attributed to "probable overfishing," the danger is not of a serious and permanent nature.

The majority of the rich grounds are either exposed or sheltered during each change of the two prevailing winds in the Philippines. Pearling in Sulu is, therefore, controlled by the northeast and southwest monsoons. This condition is in itself a great natural provision that automatically regulates the activities of the pearlers.

In view of the above facts, it appears that any division of these nine beds into two sections is unnecessary. However, it may be expedient, under serious circumstances, to segregate the Tawitawi or any rich area into districts or zones, each of which is to be treated separately; that is, provisions for closed seasons should be drawn up for each district; but this arrangement would entail large expense and necessitate strict enforcement in order to be effective. I doubt if the revenue received from the industry would defray the Government expenses incurred in patrolling the zones in question. Herein lies the failure of such regulation; and regulation that cannot be strictly enforced is worse than useless, since it is human nature to attempt to evade restrictions that can be easily eluded. Poaching in the restricted zone or zones cannot be prevented, unless there is adequate Government patrol.

January 25, while returning from Sitanki and Bongao on board the *Doña Carmen*, I sighted four pearling boats operating off Cacataan Island; and the next day three yachts were noticed working in the vicinity of Siasi, Lapac, and Lugus Islands. These places are not open for pearl fishing until after January 1, 1932, according to Department Order No. 7. The violation was brought to the attention of the customs collector in Jolo. The boat owners pleaded guilty. Business necessity forced them to violate the restriction. The boats could not work on the northern beds, on account of the strong northeast monsoon; and in the shallow-water grounds around most of the islands, the waves were rough and made the water muddy or turbid, preventing the divers from seeing the shells. During November, December, and January the pearlers were practically idle. It is obvious, therefore, that for economic reasons the boats worked wherever and whenever possible. I believe that there have been other offenses against Department Order No. 7 since January 1, 1929. The closed area is fairly large, with numerous islands and islets where clandestine pearling could be carried on with impunity, due to the inadequate means of enforcement.

The nine pearl beds in the Sulu Archipelago could be opened. The region from Basilan to Alice Channel is sufficiently extensive to sustain the operation of a large pearling fleet and the activities of the naked divers, who are confined entirely to the shallow-water grounds which seem inexhaustible. In the deeper waters there are undiscovered reserve pearl banks. The natural supply is maintained by the regulatory influence of the monsoons, which brings about rotation in the working of the pearl beds. The Sulu pearl banks are in no immediate danger of exhaustion.

The introduction of powered boats with motor-driven pumps would be dangerous to the fisheries, but, at present, of the twenty-four vessels in the pearling fleet engaged in Sulu waters only five are of this type. It is doubtful that this number will increase rapidly to such an extent as to threaten the stability of the fisheries, since a yacht so equipped costs from 8,000 to 10,000 pesos and its running expenses are comparatively high.

EXPENSES FOR OPERATING PEARLERS

The following statements were furnished me by Mr. Kashiwagi, president of the Sulu Pearling Co., Inc., and Mr. Schuck, owner of the *Rene*.

Expenses for operating a motor yacht with motor pump.

[For October, 1929.]

	Pesos.
Tender wage	45.00
Sailors wage	87.00
Oto wage (spare sailor)	7.00
Engineer wage	35.00
Shell wage paid to diver	180.18
Diver wage, monthly bonus	30.00
Market expenses	60.00
Rice, 4 sacks	43.00
Gin and tobacco	7.50
Medicine for crew	2.75
Dress, 1 pc. (diving)	140.00
Patch cloth for patching dress	15.00
Diver stocking, 1 pair	8.50
Miso and soy	4.00
Life line, 1¾-inch abacá rope	18.70
Lamp	2.10
Kerosene oil for machine	114.30
Gasoline oil and motor oil	18.00
Expenses to machine repairing	10.80
Bucket and rice pot	9.40
Total	838.23

Expenses for operating a motor yatch with motor pump—Continued.

[For November, 1929.]

	Pesos.
Market expenses	56.00
Rice, 4 sacks	43.00
Miso and soy	3.70
Gin and tobacco	7.80
Kerosene oil	108.00
Gasoline oil and motor oil	20.00
Tender wage	45.00
Sailors wage, 5 men	86.00
Oto wage	7.00
Engineer wage	35.00
Diver salary	30.00
Boarding fee at spring time	20.00
Medicine	2.00
Diving hose, 1 length, 9 fathoms (54 feet)	65.00
Marine rope	3.40
Munt metal and copper tuck	12.50
Canvas and twine	18.60
Patching cloth to repair dress of diver	10.00
Shell bonus paid to diver	79.00
Total	642.00

[For December, 1929.]

	Pesos.
Internal-revenue tax	36.87
Kerosene oil	113.00
Gasoline and motor oil	20.00
Rice, 4 sacks	44.00
Miso and soy	4.50
Medicine to crew	2.00
Gin and tobacco	7.50
Rope, 4-inch abacá, half coil	28.50
Tender wage	45.00
Sailors wage, 5 men	86.00
Oto wage	7.00
Engineer wage	35.00
Diver bonus for the month	30.00
Diver boarding expenses	20.00
Christmas bonus to sailors	35.00
Christmas bonus to divers	45.00
Market expenses, fish and vegetables	58.00
Fishing line	1.50
Leather and marine rope	3.70
Paint and painting oil	8.90
Cooking wear; dish, spoons, pots, etc.	4.80
Shell wage to diver	156.20
Total	792.48

Expenses for running a sailing boat with hand pump.

[For October, 1929.]

	Pesos.
Market expenses, fish and vegetables, etc.	58.00
Miso and soy	3.50
Kerosene oil	1.50
Pump oil, olive oil, pure	2.00
Gin and tobacco	6.00
Medicine for crew	2.70
Rice, 4 sacks	44.00
Marine rope	2.75
Tender wage monthly	40.00
Sailors wage, 5 men	85.00
Oto wage	8.50
Diver boarding fee, 14 days in harbor	15.00
Lamp	2.10
Fishing line	1.70
Life line, 1¾-inch abacá rope	13.50
Cap leather, 1 set (used for air pump)	32.00
Patching cloth for diving dress, 2 yards	15.00
Diving dress	135.00
Shell wage paid to diver	43.60
Diver salary	30.00
Total	541.85

[For November, 1929.]

	Pesos.
Market expenses	57.00
Rice, 4 sacks	44.00
Miso and soy	4.50
Gin and tobacco	7.80
Pump oil, olive oil, 2 kilograms	2.50
Kerosene oil	1.70
Oar	2.00
Sailors wages, 5 men	88.00
Oto	7.50
Tender wage	40.00
Diver wage	30.00
Diver boarding expense at harbor	20.00
Anchor and shackle	18.60
Diving hose, one length, 9 fathoms (54 feet)	60.00
Shell wage to diver	63.80
Loss advance money to sailors, not returned	12.00
Medicine to crew	3.40
Total	462.80

Expenses for running a sailing boat with hand pump—Continued.

[For December, 1929.]

	Pesos.
Internal revenue tax	13.37
Market expenses	56.00
Rice, 4 sacks	44.00
Miso and soy	4.00
Pump oil	2.00
Fishing line	1.50
Patching cloth for diving dress, 2 yards	15.00
Gasoline, oil, and rubber-making solution	8.60
Diver salary	30.00
Diver boarding expense	20.00
Sailors salary, 5 men	87.00
Oto	7.00
Tender salary	40.00
Medical treatment expenses for crew	7.60
Bonus to crew	27.60
Tender bonus	10.00
Bonus to diver	40.00
Leather and lead, making diving shoe	17.50
Shell wage to diver	121.10
Total	542.02

Expenses per month for operating the "Rene," a pearler of 10.59 gross tons, equipped with sails and hand pump. Owned by Mr. Julius Schuck.

	Pesos.
Crew, 5 men	75
One tender	45
One supercargo	25
Miscellaneous expenses for crew	50
Rice, 6 sacks	66

One diver receives 10 pesos per picul of shells and 15 per cent of the pearls.

Average monthly expenses for running the *Rene*, 300 to 400 pesos.

THE PEARLING FLEET

The pearling yachts registered in Jolo are as follows: *Aki* (formerly *Rosario*), owned by Cañizares Cheong, now run by Matsui; *Shoun*, *Kotohira*, *Alexandra*, and *Cleopatra*, owned by the Sulu Pearling Co., Inc.; *Yamato*, owned by the Ohta Development Co.; *Inari*, owned by Salim Abubakar; *Englee*, owned by Stephen Jurika; *Nachimura*, owned by Chua Hock An; *Patholbab* (*Rasidia*), owned by Mora Kum Bu; *Rene*, owned by Julius Schuck; *Cherry* and *Yablogal Morad* (*Morad*), owned by P. J. Moore; *Sirena*, owned by Mora Go Tong.

The following pearlers are registered in Zamboanga: *Kumano*, owned by the Sulu Pearling Co., Inc.; *Koun*, *Happy*, and *Sumiyoshi*, owned by the Ohta Development Co.; *Thistle*, owned by James J. Wilson; *Mindanao*, owned by Cañizares Cheóng, at present run by Arima; *Togo* and *Alice*, owned by F. Barrios, now run by Maehara; *San Francisco*, owned by F. Barrios; *Nautilus*, owned by Salim Abubakar.

These vessels are all two-masted yachts and vary in size from 6 to 14 tons. Five are equipped with Scripps driving motor and C. E. Heinke motor pump. These are *Aki*, *Cleopatra*, *Koun*, *Sumiyoshi*, and *Thistle*. The rest of the fleet use Heinke hand pump.

The boats carry a crew of from seven to thirteen men, as follows: Captain, tender, one or two divers, four or five sailors, one or two cooks, and two engineers. Each vessel is equipped with one complete diving gear, consisting of armor, pump, tubes or rubber hoses, weights, etc. The terms upon which the crew are engaged are not standardized. The following shows the varying wage scale:

	Pesos per month.
Captain	25–50
Tender	25–45
Sailor	15–20
Diver (The diver gets a bonus of 10 pesos per picul of shells and 15 to 20 per cent of the pearls.)	15–30

Under ideal conditions, a fairly well-constituted armored diver can make from two to three dives in an hour. The duration of the dive varies, since it primarily depends upon the constitution of the diver, the depth of the water, and the drift of the current. An experienced armored diver can remain under shallow water for almost an indefinite period, but in pearl fishing the practice is for the diver to work from ten to twenty minutes and rest for the same length of time. The rest is taken under water, about five fathoms below the surface, to enable the diver to readjust himself before he comes up to the boat. This is necessary, because any sudden change in pressure is usually fatal to him. The dive is limited in duration; ordinarily it is between fifteen and twenty-five minutes; it rarely exceeds thirty minutes. Diving is generally done during low tide or during the slack of the high tide, wherever possible. It is apparent, therefore, that the time spent by divers in actual work per month is comparatively short. Fifty to sixty hours represent the aver-

age monthly time spent in actual diving work per lugger, which correspond to three to four hours a day for from fifteen to eighteen days a month.

It is on record that during 1913 nine divers died; in 1914, seven; and in 1919, five, while working under water. The chief causes are inexperience and ambition to work in deep water, heart disease, syphilis, diver's paralysis, and accident through broken coupling. So far as known, no armored diver has suffered from the attack of sharks. Moro skin divers are occasionally bothered by these animals, but are never afraid of them and oftentimes emerge unscathed from the fight. No death among armored divers has been reported for 1929 and up to February 7, 1930.

PEARLS AND PEARL SHELLS OF SULU

Pearl fishing is the second most important industry in the Sulu Archipelago, which produces most of the shells and pearls for export from the Philippines. Moros, Chinese, Japanese, Christian Filipinos, and Americans are engaged in the business. The Sulu Pearling Co., Inc., and the Ohta Development Co. are the two largest pearling organizations. In Zamboanga, C. Boon Liat is the most prominent exporter of mother-of-pearl shells. There are several Chinese pearl merchants in Jolo, Siasi, Bongao, and Sitanki. The principal foreign markets for shells are Singapore and London; for pearls, Singapore, France, and London. Pearl dealers occasionally come to Zamboanga and Jolo.

During 1926, about 3,700 piculs of gold-lip pearl shells, valued at 149,166 pesos, were exported from Jolo. For 1929, the export in pearl shells amounted to 108,917 pesos. Most of these shells were sent to Sandakan, Singapore, and London. The apparent drop in the income was due to a fall in the price. At present the price of first-grade pearl shells is around 40 pesos per picul in Jolo and Zamboanga. The value of pearls obtained from Sulu waters could not be definitely ascertained, in as much as they are not all recorded. However, reliable persons estimated the total value of pearls taken in 1929 around 200,000 pesos. It was reported that last November a Moro sailor sold an 8-carat pearl to a Chinese in Jolo for 100 pesos. Tandico, one of the important Chinese merchants in the locality, bought it for 800 pesos. After a careful "doctoring," he was able to sell it to a Frenchman for 3,500 pesos. The true value of this rosy, 8-carat Sulu pearl, according to some Japanese and Chinese pearl merchants who had seen it, should be not less than 6,000 pesos.

PROTECTION OF SHELLS

The present survey has strengthened the conviction, which is held by many reliable informants, that in spite of the vigilance exercised by customs and internal-revenue officers the criminal custom of fishing undersized and immature shells is very common among skin divers as well as among armored divers. It was learned that young shells are gathered and, after being opened for the treasure they may contain, are thrown back into the water; and that divers open shells under the sea and leave them there, especially if they are undersized.

These practices are very hard to stop by governmental supervision, and can only be discontinued by the operators themselves. The operators should coöperate with the Government in protecting illegal-sized shells, for continuous indiscriminate taking of small pearl oysters would eventually bring about depletion of supply and the ruin of the industry.

The present method of measurement in limiting the minimum legal sizes for pearl shells is impractical and makes the alleged protection only apparent and not real. Shells that appear to be of legal size, but which are undersized in nacre measurement are fished and opened, only to be dumped into the sea. This is inevitable, since nacre measurement taken at right angles to the hinge joint is not feasible in practice. Divers work under conditions that make it impossible for them to use their power of discrimination and even if they can do so their criterion would be at best a big guess. Even on the deck of a pearler it is not easy to judge from the external dimension of a shell whether the nacre measurement is legal or not. The fact that the outer margin, or lip, varies in width, especially in young specimens, increases the percentage of probable error in shells that really need protection. It is obvious that pearl oysters are being destroyed in ignorance of their legal size, and many more will be sacrificed unless the method of protection is made practical. Legal size limit should be measured on the outside. To this end, the correlation between nacre measurement and external dimension was studied, and it has been found that *fourteen (14) centimeters, nacre measurement, taken at right angles to the hinge joint*, correspond, more or less, to *nineteen (19) centimeters, maximum outside long-axis measurement, taken at right angles to the base;* and *nine (9) centimeters, nacre measurement, taken at right angles to the hinge joint*, correspond to *eleven (11)*

centimeters, maximum outside long-axis measurement, taken at right angles to the base.

SUMMARY AND RECOMMENDATIONS

1. Pearl fishing in the Sulu Archipelago is primarily controlled by the two prevailing winds and tide drifts which occur periodically in Sulu waters. During the northeast monsoon, the northern beds cannot be profitably worked, and during the southwest monsoon, only a few of the southern banks can be successfully fished. When the tide drift is strong, the operation of pearl-shell fishing is suspended.

2. The division of the fisheries into north and south, in accordance with Department Order No. 7, has not only circumscribed the field of operation but also shortened the period of fishing, giving rise to a situation of considerable economic importance affecting both the boat owners and seamen. Upon the latter, particularly, the regulation works many individual hardships; and although it is true that individual hardships are inevitable to any readjustment, it is believed that the sacrifices in this case are not only unnecessary but unjustified. During the period of slack, the pearling crew is laid off and therefore deprived of its only means of livelihood, for these people are primarily brought up for sea life and cannot adapt themselves readily to land occupations. Consequently, they and their families are caused to suffer unnecessarily.

3. Observations indicate that there is no scarcity of shells in the Sulu pearl beds. Around the known banks, shells are obtained in waters ranging from 10 to 25 fathoms; and in waters of from 30 to 50 fathoms can be found a considerable number of pearl oysters that are rather difficult to secure; many of these remain in situ to constitute potential reserve nurseries from which the surrounding grounds are supplied.

4. The nine pearl beds in the Sulu Archipelago are extensive and in good condition, capable of sustaining the operation of a large pearling fleet.

5. At present the pearling fleet engaged in the waters of Mindanao and Sulu consists of twenty-four schooners, of which five are equipped with driving motor and motor-driven pump. It is believed that the present fleet is too small to do any serious damage to the fisheries.

6. Pearling boats do not as a rule work on the known grounds only, but as a matter of fact perform considerable prospecting.

7. New beds are being continually discovered.

8. Department Order No. 7 is a blanket restriction and has been found impractical. Its alleged protection is easily rendered futile, through lack of adequate enforcement.

9. That the continuity of pearl fisheries depends primarily upon natural events beyond the control of man is an established fact. The wind, the waves, the uncertain currents of the surrounding seas, and the geographical peculiarities of the banks influence the wanderings of the floating larvæ and the fate of spat-falls on the grounds. Therefore, it is apparent that, in the presence of these influences which alone can promote or facilitate the production of pearl oysters or cause the spawn to settle in favorable places, such an arbitrary remedy as devised in Department Order No. 7 is not only unnecessary but also unscientific.

10. In view of the above facts, it is recommended that Department Order No. 7 be rescinded.

11. It is further recommended that no protective measures for all beds or any banks within the Sulu Archipelago be adopted until after a careful study of the fisheries shall have been made.

12. It is also recommended that the regulation concerning the minimum legal sizes for pearl oysters be amended to read:

Margaritifera maxima Jameson (the gold-lip pearl shell). Nineteen centimeters, maximum outside long-axis measurement, taken at right angles to the base.

Margaritifera margaritifera Linnæus (the black-lip pearl shell). Eleven centimeters, maximum outside long-axis measurement, taken at right angles to the base.

ILLUSTRATIONS

PLATE 1

Map, showing the pearl-oyster beds of Mindanao and Sulu. (After Seale.)

PLATES 2 to 6. PHILIPPINE GOLD-LIP PEARL SHELL, MARGARITIFERA MAXIMA JAMESON

FIG. 1. Nacre measurement taken at right angles to the hinge joint.
2. Maximum outside long-axis measurement taken at right angles to the base.

PLATE 7. PHILIPPINE BLACK-LIP PEARL SHELL, MARGARITIFERA MARGARITIFERA LINNÆUS

FIG. 1. Nacre measurement taken at right angles to the hinge joint.
2. Maximum outside long-axis measurement taken at right angles to the base.

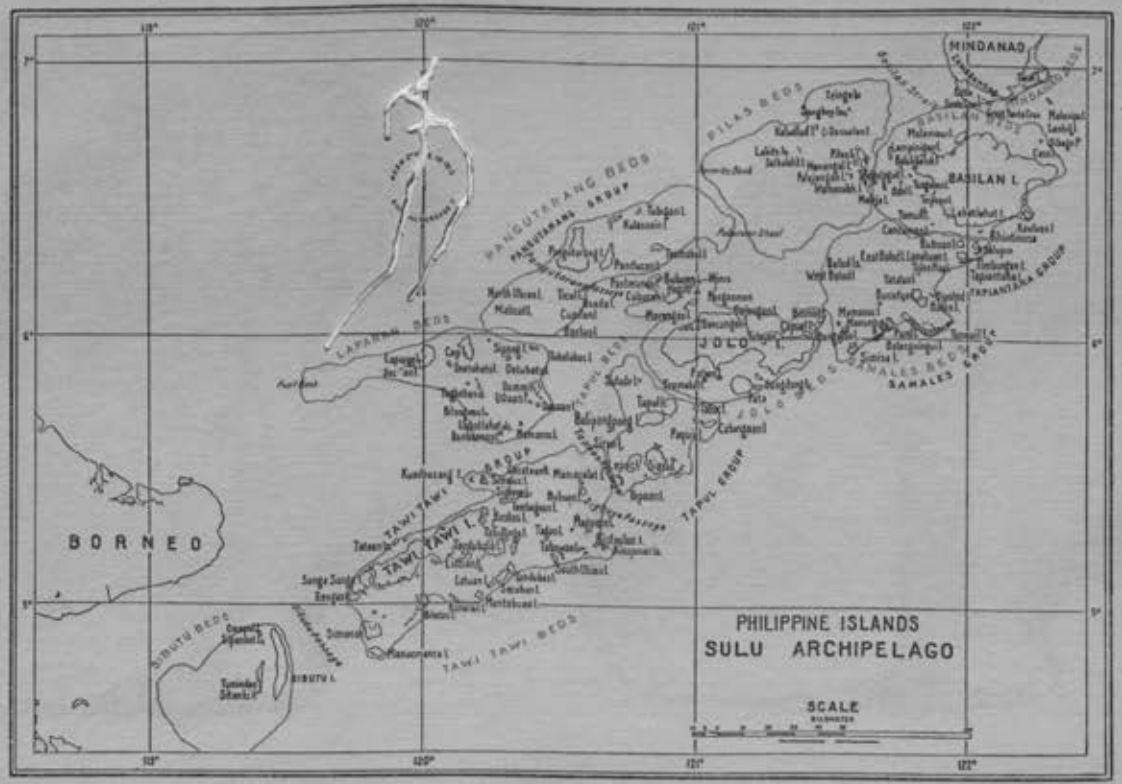
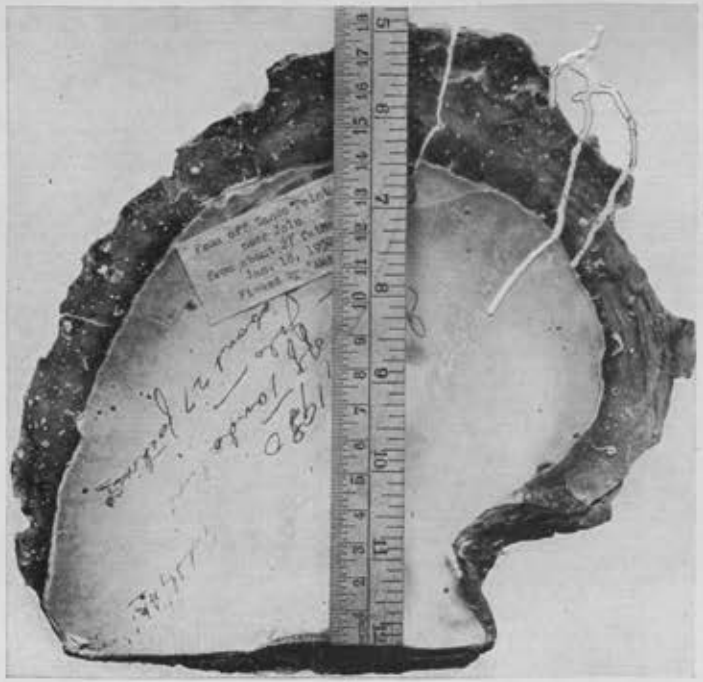


PLATE 1. THE PEARL-OYSTER BEDS OF MINDANAO AND SULU.



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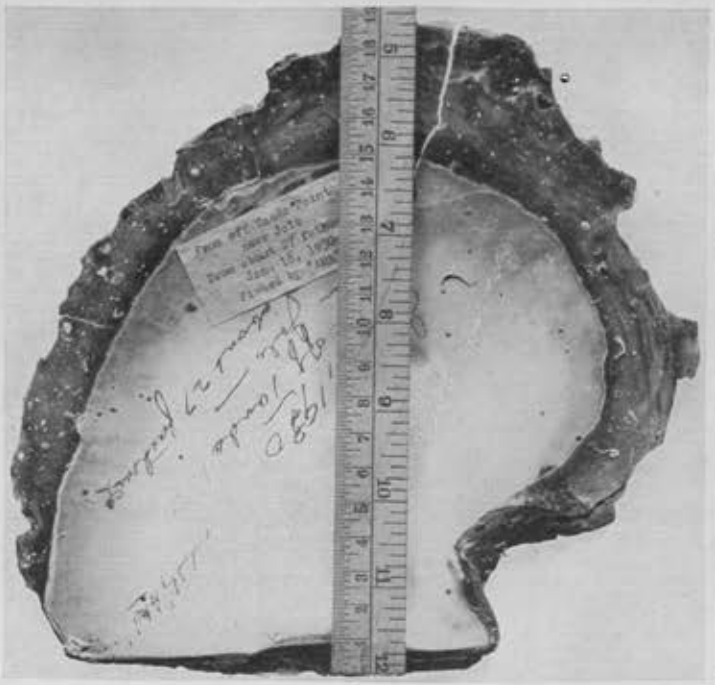

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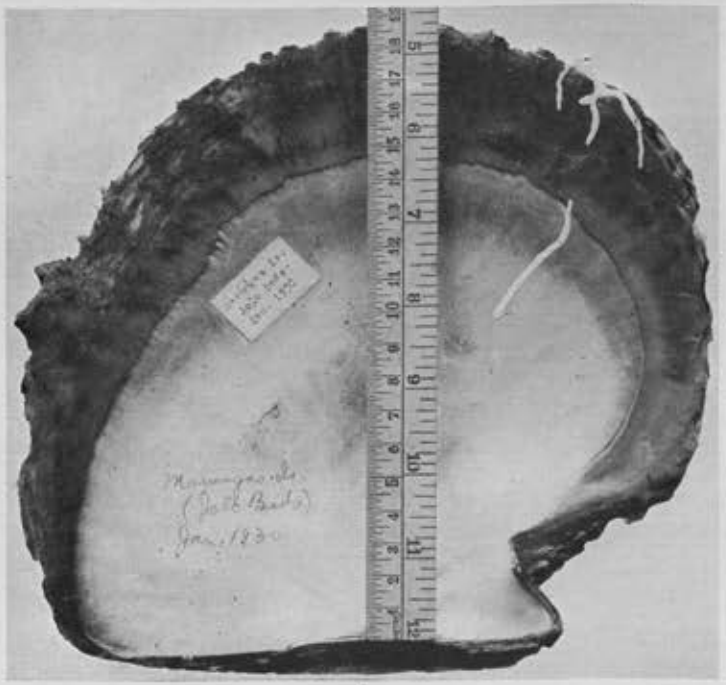
PLATE 2.



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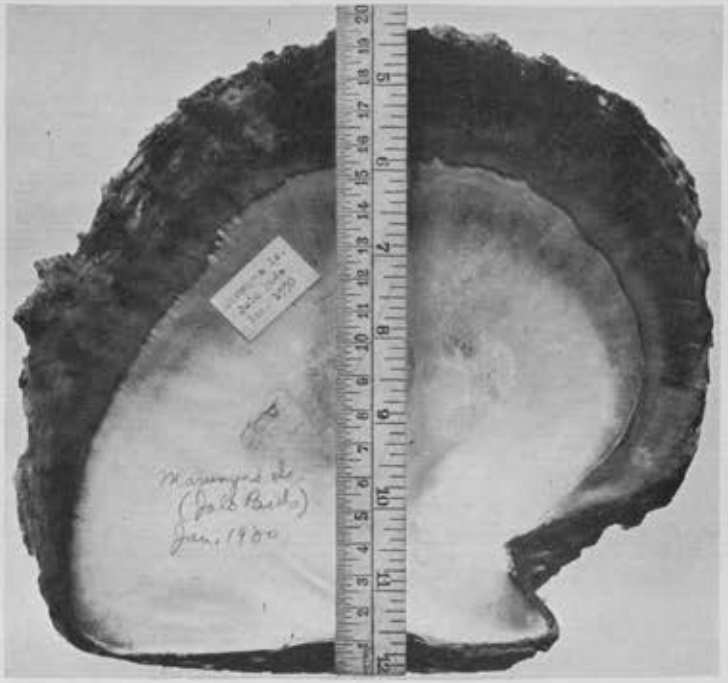

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PLATE 3.

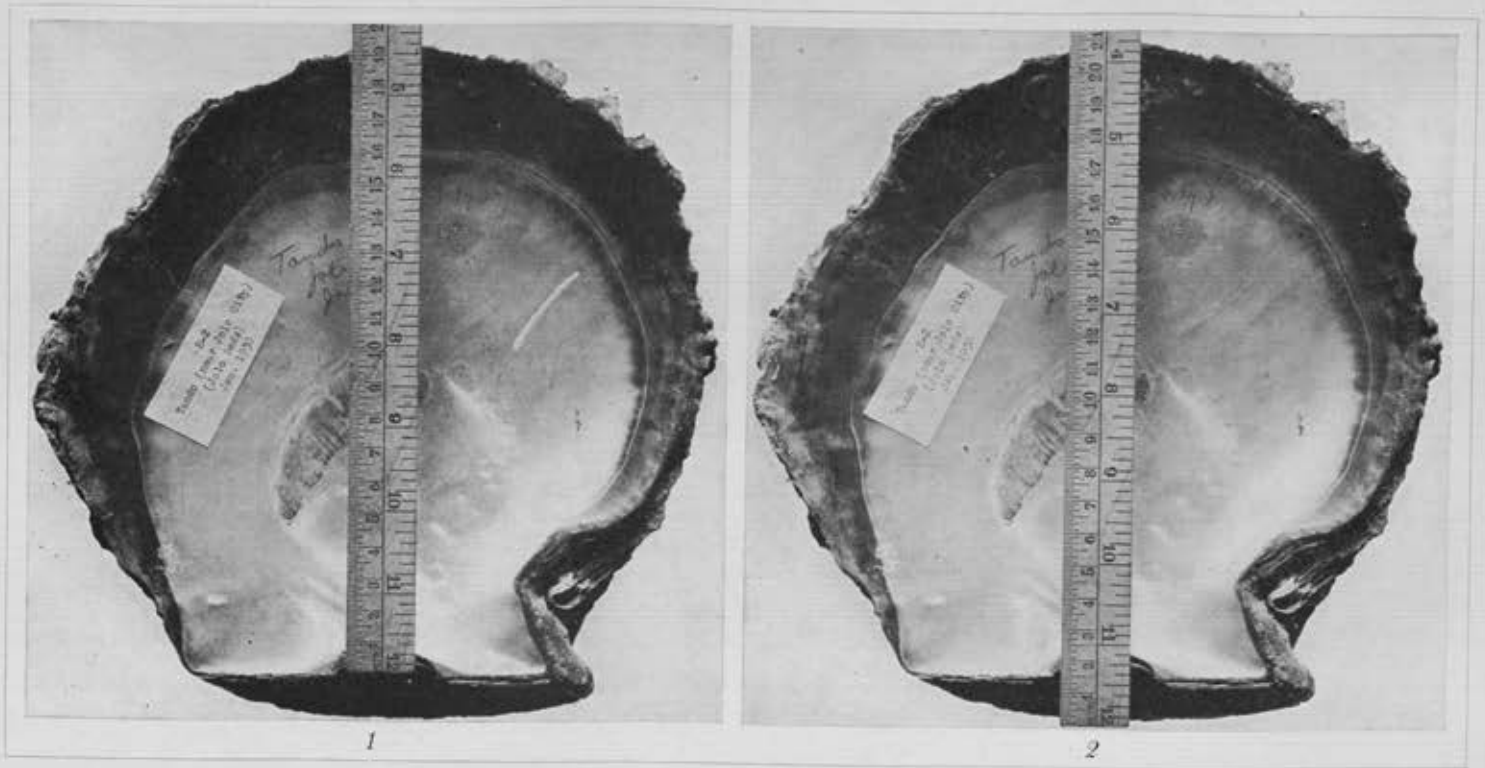

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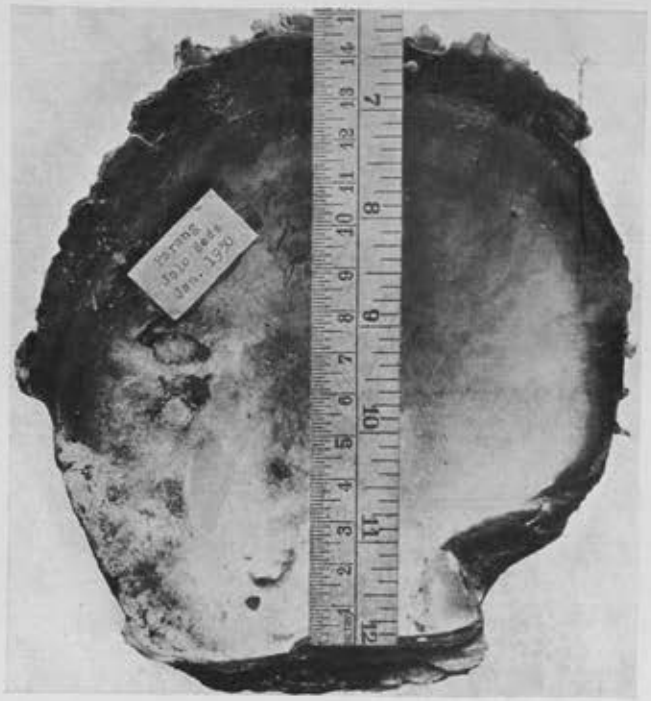
PLATE 4.



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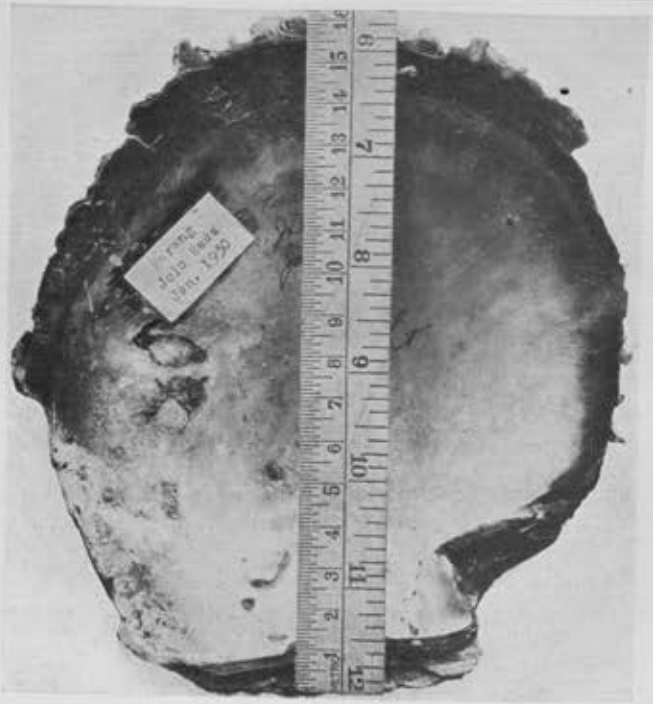

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PLATE 5.

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PLATE 6.

NOTES ON MAYON VOLCANO

By LEOPOLDO A. FAUSTINO

Chief Geologist, Division of Geology and Mines, Bureau of Science, Manila

ONE TEXT FIGURE

The eruptions of Mayon Volcano were described in considerable detail in the writer's "Mayon Volcano and its Eruptions." [1] The following notes, based on observations made during a trip to the Mayon area from March 28 to 30, 1930, for purposes other than volcanic studies, are to describe the present condition of the volcano and to record certain features of the 1928 activity that were not fully reported in the first article.

It has been consistently reported by residents of Albay that Mayon is still "smoking." During the three days that the writer was in the district no ash was noted in the steam vapors, which were issuing from the crater lip and were visible from Legaspi and other neighboring towns. The vapors rise a short distance from the crater and then disappear. The best time for observation was found to be during the early morning or late afternoon, when cumulus clouds are absent.

The summit of the volcano presents a change in appearance, though not in outline. The rock streams, which were poured from the crater and found their way through some of the notches, as well as the avalanches from above, show a striking color contrast (reddish) to masses of old material (grayish), which can be seen in spots and stand out in such relief that apparently some of the blocks are ready to roll down to a more-secure place of repose. The crater is at the present time filled with volcanic material to the brim, although being of a pasty constitution it does not taper to a point, so that viewed from kilometer 9 of the Legaspi–Libog Road the crater appears filled with a plug somewhat like a dome. Between the plug and the rim of the crater is where the steam vapors issue forth, and more steam rises near the western rim than near the eastern rim.

[1] Philip. Journ. Sci. 40 (1929) 1–43, 21 pls., 3 text figs.

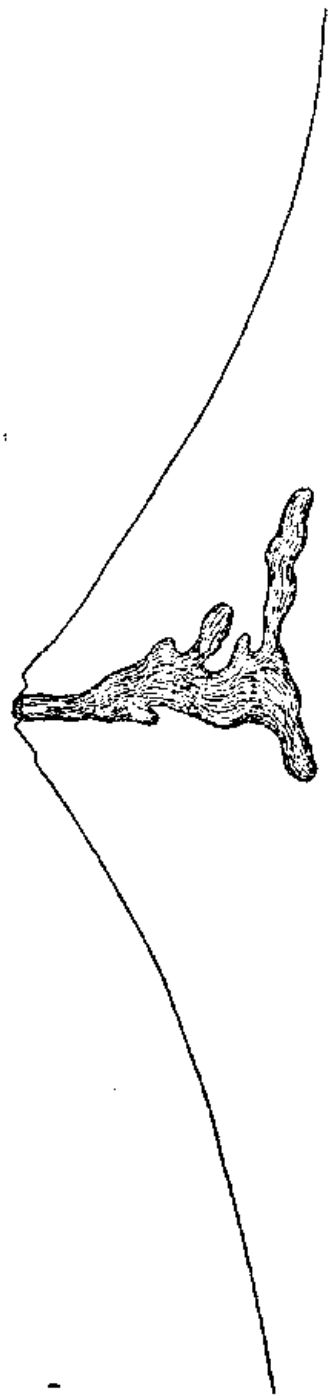

FIG. 1. Mayon Volcano, showing the direction and form of flow of the rock streams during the 1928 activity, viewed from kilometer 9, Legaspi—Libog Road.

The notch toward Libog now presents a wide open chute through which materials may come down from the crater, and it also shows the hardened volcanic dome-like plug occupying the former depression. The main rock flow from this Libog notch took the form of a ridge, which sent out several lobelike branches upon reaching the more-level topography. Rain water from the crater following the main chute has dug a narrow channel on the top of the ridge, so that there is a stream valley in the making on top of a ridge. The direction and the form of the rock flow of the 1928 activity are shown in the accompanying figure (fig. 1).

The writer did not make any calculations of the amount of material exuded during the 1928 activity in the first article as it was in the midst of the rainy season when Mayon stopped sending forth solid materials, and the outline of the rock streams could not be made out through the rain clouds. His absence in Java during 1929 was the reason for his not making the observations the following year. Based upon what can now be seen resting on the slopes, together with what the floods have taken away, it is estimated that during the 1928 activity at least

150,000,000 cubic meters of materials were exuded by Mayon Volcano. A portion of this material obstructed about a kilometer of the Legaspi-Libog Road and had to be removed. A somewhat larger portion threatened the lines of the Manila Railroad and in some parts caused considerable damage. Another portion reached Albay Gulf, and the finer materials are being redeposited along the shores. Even far-off Lake Bato, in Camarines Sur, into which a stream flowing from the higher slopes of Mayon empties, is being over-run with sandy material. According to verbal reports of Messrs. Adams and Montalban, of the division of fisheries, Bureau of Science, the bottom of Lake Bato, which the people claim was formerly mud and silt, is now very sandy.

ILLUSTRATION

TEXT FIG. 1. Mayon Volcano, showing the direction and form of flow of the rock streams during the 1928 activity, viewed from kilometer 9, Legaspi–Libog Road.

NEW OR LITTLE-KNOWN TIPULIDÆ FROM EASTERN ASIA (DIPTERA), VIII [1]

By CHARLES P. ALEXANDER

Of Amherst, Massachusetts

THREE PLATES

The crane flies discussed in the present report are almost entirely from the mountains of Formosa, the majority being from Mount Hassen. As before, this extensive series of Tipulidæ was collected by my friend Prof. Syūti Issiki, to whom my deepest thanks are extended for the opportunity of studying these flies and retaining the material in my collection. A few additional Formosan species were collected by Doctor Shiraki and Mr. Sauter, as acknowledged in the text. Two species of *Dolichopeza* were taken in the mountains of Honshiu, Japan, by Messrs. Takahashi and Ueno. A few additional species from western China, received through Mr. Herbert S. Parish, are discussed at this time. Except where noted to the contrary, the types of the novelties are preserved in my collection.

In order to supplement our scanty knowledge of the distribution of Formosan Tipulidæ, I am adding a complete list of the species taken by Professor Issiki on Hassensan, central Formosa, August 29 to 31, 1929, and October 21 to 26, 1929.

Tipulidæ from Hassensan, central Formosa, August 29 to 31 and October 21 to 26, 1929.

Dolichopeza (*Oropeza*) *shirakiella* (Alex.), 4,500 to 6,000 feet, August 30.
Tipula (*Tipula*) *yamata* Alex., 3,500 to 5,500 feet, October 22.
Limonia (*Discobola*) *margarita* (Alex.), 3,500 to 6,000 feet, August 31.
Limonia (*Discobola*) *taivanella* sp. nov., 6,500 to 7,500 feet, August 31; 7,500 feet, October 24.
Limonia (*Limonia*) *curvispina* Alex., 6,000 to 7,000 feet, August 30.
Limonia (*Limonia*) *ebriola* Alex., 5,600 feet, October 22.
Limonia (*Limonia*) *flaviterminalis* Alex., 6,000 feet, August 31.
Limonia (*Limonia*) *fraudulenta* Alex., 4,500 to 6,000 feet, August 30.
Limonia (*Limonia*) *kozinga* sp. nov., 4,500 to 6,000 feet, August 30.

[1] Contribution from the Department of Entomology, Massachusetts Agricultural College.

Limonia (*Limonia*) *remissa* Alex., 6,000 to 8,000 feet, October 23-24.
Limonia (*Libnotes*) *hassenana* sp. nov., 4,500 to 6,000 feet, August 30.
Limonia (*Rhipidia*) *formosana* (Alex.), 3,500 feet, October 24; 6,000 to 7,000 feet, August 30.
Limonia (*Rhipidia*) *triarmata* sp. nov., 4,500 to 6,000 feet, August 30.
Limonia (*Dicranomyia*) *depauperata* (Alex.), 3,500 feet, October 21.
Limonia (*Dicranomyia*) *nesomorio* (Alex.), 6,500 to 7,800 feet, October 26.
Limonia (*Dicranomyia*) *sordida* (Brunetti), 6,000 to 7,500 feet, August 30-31.
Limonia (*Dicranomyia*) *subpunctulata* sp. nov., 3,500 feet, October 21.
Limonia (*Geranomyia*) *alpestris* Alex., 6,500 to 7,800 feet, October 24.
Antocha (*Antocha*) *bifida* Alex., 3,600 feet, August 29.
Antocha (*Antocha*) *styx* sp. nov., 3,500 feet, October 24.
Helius (*Helius*) *attenuatus* Alex., 5,600 feet, October 22.
Helius (*Eurhamphidia*) *perelegans* sp. nov., 6,000 to 7,000 feet, August 30.
Thaumastoptera (*Taiwanita*) *issikiana* Alex., 5,600 feet, October 22.
Dicranoptycha issikina sp. nov., 3,500 to 5,500 feet, October 22.
Nipponomyia symphyletes (Alex.), 5,600 feet, October 22.
Tricyphona arisana Alex., 5,600 feet, October 22; 6,000 to 7,000 feet, August 30.
Tricyphona formosana Alex., 4,500 to 7,500 feet, August 30 and 31.
Dicranota (*Amalopina*) *delectata* sp. nov., 6,000 to 8,000 feet, October 23.
Dicranota (*Amalopina*) *gibbera* (Alex.), var., 4,500 to 6,000 feet, August 30.
Adelphomyia issikina sp. nov., 5,600 feet, October 22.
Epiphragma divisa Alex., 3,500 to 5,500 feet, October 22.
Pseudolimnophila autumnalis Alex., 5,600 to 7,800 feet, October 22 to 24.
Limnophila (*Prionolabis*) *serridentata* sp. nov., 3,500 to 8,000 feet, October 22 to 24.
Limnophila (*Dicranophragma*) *formosa* Alex., 6,000 to 7,000 feet, August 30.
Limnophila (*Dicranophragma*) *taiwanensis* Alex., 6,500 to 7,500 feet, August 31.
Atarba (*Atarbodes*) *fuscicornis* Edwards, 3,500 feet, October 21.
Atarba (*Atarbodes*) *leptoxantha* Alex., 6,000 to 7,000 feet, August 30.
Atarba (*Atarbodes*) *pallidicornis* Edwards, 4,500 to 6,000 feet, August 30; 3,500 to 5,600 feet, October 22.
Elephantomyia (*Elephantomyia*) *serotina* Alex., 6,500 to 7,800 feet, October 24.
Elephantomyia (*Elephantomyodes*) *uniformis* Alex., 4,500 to 6,000 feet, August 30.
Neolimnophila alticola Alex., 6,000 to 8,000 feet, October 23.
Toxorhina (*Ceratocheilus*) *taiwanicola* (Alex.), 3,500 to 7,000 feet, August 30 and 31.
Trentepohlia (*Mongoma*) *montina* sp. nov., 4,500 to 6,000 feet, August 30.

Gonomyia (*Progonomyia*) *confluenta* (Alex.), 3,500 to 5,500 feet, October 22.
Gonomyia (*Gonomyia*) *nansei* sp. nov., 3,600 feet, August 29; 2,500 to 5,600 feet, October 21 to 25.
Gonomyia (*Lipophleps*) *neonebulosa* sp. nov., 3,600 feet, August 29.
Dasymallomyia signata Brunetti, 3,500 feet, October 21.
Erioptera (*Empeda*) *liliputina* sp. nov., 3,600 feet, August 29.
Erioptera (*Empeda*) *minuscula* Alex., 3,600 feet, August 29; 3,500 feet, October 21.
Erioptera (*Ilisia*) *tenuisentis* Alex., 5,600 feet, October 22.
Ormosia anthracopoda sp. nov., 6,500 to 7,800 feet, October 26.
Molophilus arisanus Alex., 6,500 to 7,500 feet, August 31.
Molophilus nigritus Alex., 5,600 feet, October 22.
Styringomyia sinensis sp. nov., 2,500 to 3,500 feet, October 21 to 25.
Styringomyia taiwanensis sp. nov., 2,500 to 5,500 feet, October 21 to 25.

TIPULINÆ

DOLICHOPEZA (NESOPEZA) TARSALBA sp. nov.

General coloration black; antennæ short; terminal tarsal segments snowy white; wings tinged with blackish, the obliterative areas restricted; male hypopygium with the ninth tergite terminating in three blackened lobes, the median lobe long and slender; gonapophyses appearing as long, yellow, beaklike structures; eighth sternite only moderately enlarged, the caudal margin notched and bearing two pale lobes.

Male.—Length, about 11 millimeters; wing, 12.

Female.—Length, about 14 millimeters; wing, 13.

Frontal prolongation of head and palpi black. Antennæ of male much shorter than in *tarsalis;* scapal segments yellow, flagellum black; flagellar segments cylindrical, segments four to twelve of nearly equal length, the last segment about one-third the length of the penultimate; verticils shorter than the segments. Head yellowish brown, brighter in front, darkening to brown on posterior vertex and occiput.

Thorax chiefly brownish black, in the male with a paler median præscutal stripe; anterior dorsopleural region somewhat paler. Halteres obscure yellow, the knobs blackened. Legs with the coxæ blackened, their apices and the trochanters obscure yellow; femora, tibiæ, and basitarsi black, the femoral bases restrictedly pale; tips of basitarsi and remaining tarsal segments white. Wings with a strong blackish tinge, the oval stigma darker; a dark seam on anterior cord; obliterative areas very restricted, appearing as small areas before the stigma and across the fork of M; veins black, pale in the obliterative areas. Venation: Medial forks shallow.

Abdominal tergites brownish black, the basal sternites variegated with a broad, subapical, yellow annulus. Male hypopygium (Plate 2, fig. 25) relatively small. Ninth tergite (Plate 2, fig. 26) terminating in three blackened lobes, the median lobe slender, a little longer than the laterals. Gonapophyses (Plate 2, fig. 27) long and conspicuous, jutting from the genital chamber as yellow beaklike structures. Eighth sternite (Plate 2, fig. 28) with a deep U-shaped emargination that bears two small lobes.

Habitat.—Japan (Honshiu).

Holotype, male, Shirahone Hot Springs, Shinano, July 24, 1929 (*M. Ueno.*) Allotopotype, female, with the type.

Dolichopeza (Nesopeza) tarsalba is most closely allied to *D. (N.) tarsalis* (Alexander), differing most conspicuously in the short antennæ of the male and details of structure of the male hypopygium.

DOLICHOPEZA (OROPEZA) SAITAMENSIS sp. nov.

General coloration brown, the præscutum with three slightly darker subnitidous stripes; head black; wings tinged with brown, with three creamy areas, the largest beyond the stigma; male hypopygium with the caudal margin of the ninth tergite a weakly chitinized pale flange, the median portion further produced into a quadrate plate.

Male.—Length, about 12 millimeters; wing, 13.5.

Frontal prolongation of head dark brown, paler laterally; palpi black. Antennæ with the scapal segments yellow, flagellar segments passing into dark brown; segments subcylindrical, the verticils relatively short and unilaterally arranged; terminal segment about two-thirds the penultimate. Head black, sparsely pruinose.

Mesonotum brown, the præscutum with three slightly darker brown, faintly shiny brown stripes; scutal lobes and scutellum dark brown; postnotum paler, with short yellow setæ. Pleura brownish testaceous. Halteres tinged with dusky. Legs with the coxæ and trochanters testaceous; remainder of legs passing into brown, the tarsi slightly paler yellow. Wings broad, tinged with brown, the stigma darker brown; cream-colored obliterative areas before and beyond the stigma, the latter very large; a third obliterative area across the base of cell 1st M_2; veins pale brown. Venation: Cell 1st M_2 relatively long and narrow.

Abdominal segments brown, indistinctly variegated with brownish yellow. Male hypopygium with the ninth tergite

(Plate 2, fig. 29) broad, the caudal margin appearing as a pale, weakly chitinized, narrow border, with a median quadrate extension that is produced into short lateral points; sublateral points slenderer; lateral arms unusually broad, the obtuse tips microscopically roughened. Outer dististyle a relatively short clavate lobe. Inner dististyle (Plate 2, fig. 30) distinctly bilobed at apex, the outer angle being produced into a slender rod, the remainder a broad, smooth, darkened blade.

Habitat.—Japan (Honshiu).

Holotype, male, Chichibu, Saitama, May 29, 1919 (*R. Takahashi*).

Dolichopeza (*Oropeza*) *saitamensis* is closest to the species *D.* (*O.*) *bispinula* (Alexander), differing in the structure of the male hypopygium, especially the armature of the ninth tergite.

LIMONIINÆ

LIMONIINI

LIMONIA (DISCOBOLA) TAIVANELLA sp. nov.

Allied to *argus;* wings with scattered brown dots in cells R and M, in addition to the ocellate pattern; male hypopygium with the caudal margin of the tergite produced into two blackened setiferous lobes that are separated by a quadrate notch; ventral dististyle an elongate-oval lobe.

Male.—Length, 6.5 to 7 millimeters; wing, 9 to 9.5.

Female.—Length, 7.5 to 8 millimeters; wing, 9 to 10.

Rostrum and palpi black, the former about one-half the remainder of head. Antennæ black, the segments with short pale apical pedicels. Head gray, variegated with blackish, the anterior vertex more silvery gray.

Pronotum greenish yellow, narrowly darkened laterally. Mesonotal præscutum olive yellow to yellow, the lateral margins brown, the median region more infuscated; scutal lobes brown; scutellum and postnotum yellow, sparsely pollinose. Pleura yellow, whitish pruinose, with two narrow brown stripes, the more dorsal extending from behind the fore coxæ, completely suffusing the pleurotergite; ventral stripe occupying the ventral sternopleurite; dorsopleural region dark, connected with the areas on the propleura and lateral margins of præscutum. Halteres black, the extreme base of stem and apical half of knob whitish. Legs with the coxæ and trochanters yellow; femora brownish yellow, the distal end light yellow, inclosing a narrow black subterminal ring that is nearly equal in width to the yellow apex; in some specimens, the basal portion of the femora is clearer yellow;

remainder of legs brownish yellow, the terminal tarsal segments passing into brownish black. Wings (Plate 1, fig. 1) yellow, with a brown ocellate pattern that is much as in *argus;* in addition, with scattered brown dots in cells R and M, these lacking in *argus.*

Abdominal tergites brown, the extreme caudal margins pale, in cases with a more yellow basal and medial ring on each segment; sternites greenish yellow, the caudal margins of the segments narrowly dark brown; hypopygium yellow. Male hypopygium (Plate 2, fig. 31) with the tergite, 9*t*, narrowed apically, each lateral angle produced into a dusky setiferous lobe, these separated by a quadrate notch. Ventromesal lobe of basistyle, *b*, very stout, occupying almost the entire face of the style. Ventral dististyle, *vd*, an elongate-oval pale lobe that is approximately twice as long as the dorsal dististyle; rostral prolongation slender, darkened, the two pale peglike spines arising from face of style above. Dorsal dististyle with the surface microscopically roughened. Gonapophyses, *g*, with the apex of mesal apical lobe bluntly obtuse.

Habitat.—Formosa.

Holotype, male, Hassensan, altitude 6,500 to 7,500 feet, August 31, 1929 (*S. Issiki*). Allotopotype, female, altitude 7,500 feet, October 24, 1929 (*S. Issiki*). Paratopotypes, 2 males; paratype, 1 female, Shōrei, altitude 7,000 to 8,000 feet, October 25, 1928 (*S. Issiki*).

Limonia (*Discobola*) *taivanella* is separated from *L.* (*D.*) *argus* (Say) chiefly by the very different structure of the male hypopygium. The female from Shōrei had earlier been recorded as *argus*.[2] The Arisan record for *argus* in the preceding part under this general title[3] is correct, and there are unquestionably three species of *Discobola* inhabiting the higher mountains of Formosa.

LIMONIA (LIMONIA) KOXINGA sp. nov.

General coloration of thorax reddish yellow, unmarked; head blackish gray; halteres relatively short, brown; legs yellow, the femoral and tibial tips narrowly blackened; wings gray, darker basally, stigma brown; narrow dark seams along cord and outer end of cell 1st M_2; male hypopygium with the tergite deeply notched; dorsal dististyle lacking; rostral prolongation of ven-

[2] Philip. Journ. Sci. 40 (1929) 526.
[3] Philip. Journ. Sci. 42 (1930) 509.

tral dististyle long and slender; a spine arising from a long basal tubercle on face of dististyle near base; tergal valves of ovipositor very small.

Male.—Length, about 7.5 to 8 millimeters; wing, 7 to 7.4.

Female.—Length, about 8.5 to 9 millimeters; wing, 7.5 to 7.6.

Rostrum shiny black; palpi brownish black. Antennæ light brown; flagellar segments oval, becoming more slender and elongate outwardly, the verticils a little exceeding the segments; terminal segment a trifle shorter than the penultimate. Head blackish gray.

Pronotum reddish yellow, more blackened anteriorly above. Thorax uniformly reddish yellow, the surface nitidous, the pleura clearer yellow. Halteres relatively short, brown, the base of the stem yellow. Legs yellow, the femoral tips narrowly but conspicuously blackened; tibiæ more narrowly darkened; tarsi gradually darkened. Wings (Plate 1, fig. 2) slightly tinged with gray, the basal cells somewhat more strongly so; cells C and Sc more yellowish; stigma brown; narrow dark seams along cord and outer end of cell 1st M_2; veins black. Venation: Sc_1 ending opposite or just beyond midlength of Rs, Sc_2 at its tip; free tip of Sc_2 and R_2 about in alignment; m-cu at or close to the fork of M.

Abdominal tergites dark brown, the caudal margins narrowly pale; sternites and hypopygium paler. Male hypopygium (Plate 2, fig. 32) with the tergite, *9t*, deeply notched medially, the conspicuous lateral lobes with long conspicuous setæ. Basistyle, *b*, with the ventromesal lobe conspicuous. Ventral dististyle, *vd*, large and fleshy, much larger than the basistyle; rostral prolongation long and slender, curved; a single powerful spine arises from the face of the ventral dististyle near base of prolongation, this spine from a longer basal tubercle. No dorsal dististyle. Gonapophyses, *g*, with the mesal apical angle elongate, gradually expanded at tip into a weak spatula. Ovipositor with the tergal valves (cerci) very small.

Habitat.—Formosa.

Holotype, male, Hassensan, altitude 4,500 to 6,000 feet, August 30, 1929 (*S. Issiki*). Allotopotype, female. Paratopotypes 1 male, 1 female.

Limonia koxinga is named from Koxinga, piratic lord of Taiwan in the seventeenth century. The species is very different from *L.* (*L.*) *alticola* (Edwards) in the details of struc-

ture though very similar in the reduced tergal valves of the ovipositor.

LIMONIA (LIBNOTES) HASSENANA sp. nov.

Female.—Length, about 7 millimeters; wing, 7.5.

Generally similar and closely allied to *L.* (*L.*) *riverai* Alexander (Luzon), differing especially in the details of venation.

General coloration dark blackish gray. Halteres darkened, the base of the stem brightened. Legs with the femora obscure yellow, scarcely darkened at tips. Wings (Plate 1, fig. 3) with Rs long and gently arcuated; Sc ending opposite r-m and thus appearing very long, the distance between origin of Rs and Sc_2 subequal to R_{2+3} and exceeding R_3 alone; free tip of Sc_2 some distance before R_2, the latter arcuate. In the absence of the male, the length of the costal fringe in this sex cannot be stated.

Habitat.—Formosa.

Holotype, female, Hassensan, altitude 4,500 to 6,000 feet, August 30, 1929 (*S. Issiki*).

LIMONIA (RHIPIDIA) TRIARMATA sp. nov.

General coloration gray; antennæ with eight flagellar segments, each bearing two branches; terminal segment oval; wings gray, variegated with white spots and dots, the cubital and anal fields more uniformly gray; Sc_1 ending about opposite one-third the length of Rs; male hypopygium with three long spines on rostral prolongation of ventral dististyle.

Male.—Length, about 6 millimeters; wing, 5.5.

Rostrum and palpi black. Antennæ with flagellar segment one merely produced beneath, segments two to nine inclusive with two branches, the longest about twice the segments; flagellar segments ten and eleven merely produced; terminal segment relatively short, oval; basal enlargements and branches dark, the long glabrous apical necks pale, these necks shortening on outer segments, on the penultimate and antepenultimate very short. Head brownish gray.

Thorax gray, the præscutum with a median brown stripe, the lateral stripes lacking or nearly so. Halteres pale, the knobs weakly infuscated. Legs brown, the segments not conspicuously variegated. Wings (Plate 1, fig. 4) gray, variegated with white spots and dots, most evident in the radial and medial fields, the cubital and anal fields almost uniformly darkened; veins brownish black. Venation: Sc_1 ending about opposite one-third the length of Rs, Sc_2 not far from its tip; m-cu before the fork of M.

Male hypopygium (Plate 2, fig. 33) much as in *maculata;* rostral prolongation of ventral dististyle, *vd*, unusually long and slender, at near midlength with three long reddish spines from a restricted point, these spines a little shorter than the prolongation.

Habitat.—Formosa.

Holotype, male, Hassensan, altitude 4,500 to 6,000 feet, August 30, 1929 (*S. Issiki*).

Limonia (Rhipidia) triarmata is evidently closely allied to *L. (R.) maculata* (Meigen) and may prove to be a geographic race of the latter. I have seen a closely allied species or race from Szechwan, China.

LIMONIA (DICRANOMYIA) SUBPUNCTULATA sp. nov.

Allied to *punctulata;* wings without spots in costal and subcostal cells except at ends of cells; male hypopygium with the rostral prolongation of the ventral dististyle elongate, with two small basal spines; gonapophyses simple at tips.

Male.—Length, about 5 to 5.3 millimeters; wing, 6.

Female.—Length, about 6.5 to 7 millimeters; wing, 6.5.

Rostrum and palpi black. Antennæ black; flagellar segments oval, gradually decreasing in size outwardly. Head dark gray; anterior vertex narrow.

General coloration of thorax gray, the præscutum with a broad median brown stripe and less distinct lateral stripes; scutellum light gray. Halteres dusky, the knobs dirty white. Legs with the coxæ dark brown, pruinose; trochanters brown; femora gradually deepening to dark brown, the extreme tips narrowly obscure yellow; tibiæ and tarsi brownish yellow, the latter blackened at tips. Wings (Plate 1, fig. 5) cream-colored, with a restricted brown pattern, including spots at arculus; tip of Sc; R_2; at intervals along cord; outer end of cell 1st M_2 and tip of R_3; small spots at two-thirds the length of R_{4+5}, midlength of distal section of M_{1+2}, midlength of M, and two spots in cell 1st A adjoining vein 2d A; cells C and Sc without darkening except at each end of cells; veins yellow, dark brown in the infuscated areas. Venation: Sc, ending opposite origin of Rs, Sc_2 at tip; Rs straight, oblique; cell 1st M_2 elongate; m-cu at fork of M.

Abdomen dark gray, the caudal margins of the segments paler; basal sternites obscure yellow; hypopygium dark. Male hypopygium (Plate 2, fig. 34) with the lateral lobes of the ter-

gite, *9t*, low and obtuse, with numerous setæ. Basistyle, *b*, relatively small, the ventromesal lobe large. Ventral dististyle, *vd*, a large fleshy lobe, the rostral prolongation long and slender, at base with two small subequal spines that are less than one-half as long as the prolongation. Dorsal dististyle a gently curved chitinized rod, narrowed to the acute tip. Gonapophyses, *g*, with the mesal apical angle a simple acute spine.

Habitat.—Formosa.

Holotype, male, Meizi Hot Springs, foot of Hassensan, altitude 2,500 feet, October 25, 1929 (*S. Issiki*). Allotopotype, female, October 26, 1929 (*S. Issiki*). Paratopotypes, 20 males and females, with the type; paratypes, 1 male, Hassensan, altitude 3,500 feet, October 21, 1929 (*S. Issiki*); 1 male, Nōkō, altitude 8,000 feet, June 26, 1927 (*S. Issiki*).

Limonia (*Dicranomyia*) *subpunctulata* belongs to the *punctulata* group and has been confused with *punctulata* (de Meijere). The latter is figured by de Meijere as having a single rostral spine with its tip strongly curved; *L.* (*D.*) *fullowayi* (Alexander) has the rostral spine single, entirely straight, as long as the prolongation itself or longer. Gonapophyses with the mesal apical angle blackened, broad, more or less toothed.

LIMONIA (GERANOMYIA) APICIFASCIATA sp. nov.

General coloration reddish brown; rostrum black, the apical fourth yellow; halteres dusky; legs yellow; wings whitish hyaline with a heavy brown, chiefly costal pattern, the outermost area a complete fascia; male hypopygium with the two rostral spines arising from a long slender common tubercle.

Male.—Length, excluding rostrum, about 6 millimeters; wing, 6.8; rostrum, about 3.

Rostrum black, the apical fourth paling to yellow; palpi black. Antennæ with the scapal segments black; flagellum pale brown; flagellar segments with short inconspicuous verticils. Head gray, the anterior vertex more silvery, the posterior vertex more blackish.

Mesonotal præscutum brown, with four reddish brown stripes, the lateral portions more pruinose; scutum and scutellum pruinose, each scutal lobe with an elongate-triangular reddish brown area; postnotum pale brown, the surface pruinose. Pleura testaceous yellow. Halteres dusky, the base of the stem restrictedly yellow. Legs with the coxæ and trochanters yellowish testaceous; remainder of legs yellow, only the terminal tarsal

segments infumed. Wings (Plate 1, fig. 6) whitish hyaline, the costal region pale yellow; a heavy brown, chiefly costal pattern; six major costal areas that extend into the cells behind, the third at origin of Rs, the last a complete, transverse, nearly apical fascia; between the major areas in cells C and Sc are smaller dark spots that restrict the ground color to small areas; additional restricted brown areas along cord and outer end of cell 1st M_2 and as marginal clouds at ends of longitudinal veins and at midlength of cell 2d A; veins yellowish brown, darker in the infuscated areas. Venation: Sc long, Sc_1 opposite the fork of Rs, Sc_2 at its tip; a supernumerary crossvein in cell Sc; m-cu at fork of M; vein 2d A short, strongly curved to margin, the cell wide.

Abdominal tergites reddish brown, more blackened medially and subapically, the caudal margin narrowly pale; sternites more uniformly yellow; hypopygium brownish yellow. Male hypopygium (Plate 2, fig. 35) with the tergite, *9t*, transverse, the caudal margin very gently emarginate. Basistyle, *b*, much smaller than the ventral dististyle, the ventromesal lobe large. Ventral dististyle, *vd*, oval, the rostral region large, produced into a very long tubercle that bears two slightly longer reddish spines. Dorsal dististyle a strongly curved sickle. Gonapophyses, *g*, with the mesal apical angle a strongly curved subacute spine.

Habitat.—Formosa.

Holotype, male, Shinten, December 3, 1928 (*S. Issiki*).

Limonia (*Geranomyia*) *apicifasciata* is very different from allied regional species of the subgenus.

ANTOCHA (ANTOCHA) STYX sp. nov.

General coloration dark gray; halteres and legs blackened; wings tinged with blackish; male hypopygium black, the outer dististyle pointed at apex; ædeagus broad.

Male.—Length, about 4.5 to 5 millimeters; wing, 5 to 5.5.

Rostrum and palpi black. Antennæ black; flagellar segments oval, gradually decreasing in size outwardly, the terminal segment about one-third longer than the penultimate; verticils very short, more conspicuous on basal and two outer segments. Head dark gray.

Mesonotum dark gray, the præscutum almost covered by still darker confluent stripes. Halteres infuscated, especially the knobs. Legs black. Wings (Plate 1, fig. 7) with a strong

blackish tinge; stigma a little darker; veins still darker brown. Venation: Sc_1 ending a short distance before the fork of the long Rs; cell 1st M_2 closed.

Abdomen blackish gray, the hypopygium black. Male hypopygium (Plate 2, fig. 36) with the caudal margin of the tergite, *9t*, gently emarginate. Surface of basistyle, *b*, with very numerous course setæ. Outer dististyle chitinized, curved gently to an acute point, the face carinate. Inner dististyle very strongly curved. Ædeagus, *a*, broad.

Habitat.—Formosa.

Holotype, male, Meizi Hot Springs, foot of Hassensan, altitude 2,500 feet, October 26, 1929 (*S. Issiki*). Paratopotypes, 3 males, October 25, 1929 (*S. Issiki*); paratypes, 2 males, Hassensan, altitude 3,500 feet, October 24, 1929 (*S. Issiki*).

Antocha styx is readily told from all described regional species by the diagnostic features listed above.

HELIUS (EURHAMPHIDIA) PERELEGANS sp. nov.

Female.—Length, about 5.5 millimeters; wing, 5.5.

Allied to *H. (E.) inelegans* Alexander, differing especially in the venational details and pattern of the legs.

Mesonotal præscutum with the disk almost covered by three confluent brown stripes, restricting the ground color to the humeral and lateral portions; scutal lobes dark brown. Femora with the tips rather broadly and conspicuously snowy white; tibial bases narrowly pale, the tips very broadly white; tarsi white, the terminal tarsal segments darkened. Wings (Plate 1, fig. 8) with Sc short, Sc_1 ending opposite r-m, Sc_2 a little longer than Sc_1; Rs short and straight; m-cu before the fork of M.

Habitat.—Formosa.

Holotype, female, Hassensan, altitude 6,000 to 7,000 feet, August 30, 1929 (*S. Issiki*).

DICRANOPTYCHA ISSIKINA sp. nov.

General coloration of thorax brown, the præscutum with four narrow shiny black stripes, the scutal lobes further variegated with similar areas; legs yellow; wings gray, the prearcular and costal regions clear yellow; stigma elongate, brown; veins yellow, narrowly bordered on the membrane by the same color.

Male.—Length, 8.5 to 9.5 millimeters; wing, 9 to 10.5.

Rostrum brownish gray; palpi dark brown. Antennæ reddish brown, the first segment darker at base; basal flagellar segments with long conspicuous verticils, these shorter on the outer segments. Head dark brownish gray.

Mesonotal præscutum grayish brown to brown, more grayish laterally, with four narrow and incomplete shiny black stripes, the intermediate pair longer and broader; scutum brownish gray, each lobe with two shiny black areas; scutellum brownish gray, the postnotal mediotergite clearer gray. Pleura gray. Halteres yellow, the knobs a little dusky. Legs with the coxæ and trochanters brownish yellow; remainder of legs clearer yellow, with dark setæ, the outer tarsal segments darkened. Wings (Plate 1, fig. 9) gray, the prearcular and costal regions clear yellow; stigma elongate, dark brown; veins yellow, narrowly bordered on membrane by clear yellow. Venation: Rs angulated and short-spurred at origin, longer than cell 1st M_2; m-cu at before midlength of the latter.

Abdominal tergites obscure yellow, with a dorsomedian brown line; sternites clearer yellow; a conspicuous subterminal blackish gray ring involving segments six to eight; hypopygium fulvous. Male hypopygium (Plate 2, fig. 37) with the tergite transverse, the ventrolateral angles produced caudad into spatulate pale blades (lateral processes). Outer dististyle, *od*, terminating in a slender black point, the surface with abundant erect yellow setulæ, the disk and inner margin with longer recurved setæ. Inner dististyle, *id*, longer, set with spinous setæ. Gonapophyses small, slender, shorter than the bifid ædeagus, *a*.

Habitat.—Formosa.

Holotype, male Hassensan, altitude 3,500 to 5,500 feet, October 22, 1929 (*S. Issiki*). Paratopotype, male.

I take great pleasure in naming this beautiful *Dicranoptycha* in honor of the collector, Prof. Syūti Issiki, distinguished student of the Mecoptera of eastern Asia. The species is very distinct from all other members of the genus so far discovered in eastern Asia.

PEDICIINI

DICRANOTA (AMALOPINA) DELECTATA sp. nov.

General coloration pale yellow, including the legs and halteres; wings cream yellow, with a conspicuous brown pattern that includes the prearcular cells and darkened costa as far distad as the origin of Rs; supernumerary crossveins in cells R_1 and R_3; cell 1st M_2 closed.

Male.—Length, about 6 millimeters; wing, 7.

Rostrum and palpi brown. Antennæ 14-segmented; basal segment black, the remaining segments pale testaceous brown. Head ocherous, the vertex a little darkened.

Mesonotal præscutum pale yellow, with a whitish bloom; scutal lobes more darkened; scutellum and postnotum pale yellow, with a whitish bloom. Pleura pale yellow. Halteres yellow. Legs yellow, the two terminal tarsal segments brown. Wings (Plate 1, fig. 10) pale cream yellow, with a conspicuous brown pattern; prearcular region and cells C and Sc darkened to approximately opposite the origin of Rs; base of cell R darkened to opposite Sc_2; stigmal region diffusely darkened; narrow but conspicuous dark brown seams at origin of Rs, along cord and outer end of cell 1st M_2, on R_2 and the supernumerary crossvein in cell R_2, at the tips of veins R_{1+2} and R_2, and at fork of M_{1+2}; veins yellow, brown in the infuscated areas. Venation: A supernumerary crossvein in cell R_1; Rs strongly arcuated at origin; a supernumerary crossvein in cell R_2 more than its own length beyond R_2; cell 1st M_2 closed.

Abdomen with the basal segments yellow, ringed caudally with brown, the amount increasing on the outer segments; outer segments, including the hypopygium, more uniformly dark brown.

Habitat.—Formosa.

Holotype, male, Hassensan, altitude 6,000 to 8,000 feet, October 23, 1929 (*S. Issiki*).

Dicranota (*Amalopina*) *delectata* is most nearly allied to *D.* (*A.*) *dicranotoides* (Alexander) and *D.* (*A.*) *sibirica* (Alexander), differing in the wing pattern and venation.

ADELPHOMYIA ISSIKINA sp. nov.

General coloration of notum ocherous, marked with brown; knobs of halteres infuscated; legs yellow, the femoral tips conspicuously blackened; wings with the costal third cream-colored, the remainder conspicuously darkened; a sparse but heavy wing pattern; abundant macrotrichia in cells of wing beyond cord; R_2 far before fork of R_{3+4}; cell M_1 present.

Female.—Length, about 5.5 millimeters; wing, 6.

Rostrum reddish brown; palpi black. Antennæ 16-segmented, black, the flagellar segments a little paler; flagellar segments becoming more slender and elongated; verticils conspicuous. Head light brown.

Pronotum dark brown, paler behind. Mesonotum ocherous to reddish brown, the præscutum with a more or less distinct median brown stripe; scutal lobes darkened; scutellum and postnotum dark brown. Pleura chiefly dark brown, the sternopleurite, meral region, and pleurotergite more yellowish. Hal-

teres pale, the knobs infuscated. Legs with the fore coxæ infuscated, the remaining coxæ and trochanters yellow; femora yellow, the tips narrowly blackened; tibiæ yellow, the tips very narrowly blackened; tarsi yellow, passing into brown; tibial spurs distinct. Wings (Plate 1, fig. 11) with the costal third cream-colored, the central and posterior thirds darkened, the anal cells again somewhat more yellowish; a heavy dark brown pattern, arranged as follows: Origin of Rs; stigma; along cord and outer end of cell 1st M_2; veins yellow, more infuscated in the darkened regions. Abundant macrotrichia in the cells of the wing beyond the cord. Venation: Sc_1 ending nearly opposite the fork of Rs, Sc_2 some distance from its tip; Rs angulated and spurred at origin; R_2 more than one-half its length before the fork of R_{3+4}; cell M_1 present; m-cu at near mid-length of cell 1st M_2; vein 2d A nearly straight.

Abdominal tergites dark brown; sternites brown basally, the caudal half obscure yellow, the amount of the latter decreasing on the outer segments. Ovipositor with the basal shields blackened; valves yellow, the sternal valves blackened ventrally.

Habitat.—Formosa.

Holotype, female, Hassensan, altitude 5,600 feet, October 22, 1929 (*S. Issiki*). Paratopotype, female.

Adelphomyia issikina is another of the very distinct species of crane flies discovered by the collector in the mountains of Formosa. I take great pleasure in dedicating the present fly to Professor Issiki who has done so much toward making known the rich tipulid fauna of the island. The present species is very distinct from all regional forms. I would believe that *Oxydiscus* de Meijere[4] is identical with *Adelphomyia*, despite the implied lack of tibial spurs, a highly variable character in this, as well as other groups of Tipulidæ.

HEXATOMINI

LIMNOPHILA (PRIONOLABIS) SERRIDENTATA sp. nov.

General coloration black, the surface opaque by a sparse pruinosity; wings grayish with vague seams on the crossveins and deflections; Sc_1 much longer than Sc_2; R_{2+3} from one-half to two-thirds R_3; cell M_1 lacking; m short and straight, less than the basal section of M_3; male hypopygium with the gonapophyses serrate along outer margin.

[4] Tijdschr. voor Entomologie 56 (1913) 350–351.

Male.—Length, about 4.3 to 5 millimeters; wing, 5 to 6.2.

Female.—Length, about 4.8 to 5.2 millimeters; wing, 5.5 to 6.

Rostrum and palpi black. Antennæ black throughout; flagellar segments oval, gradually decreasing in size outwardly, the terminal segment little larger than the penultimate; longest verticils unilaterally arranged, exceeding the segments in length. Head black, pruinose.

Thorax black, the surface pruinose, least so on the median region of præscutum. Halteres pale yellow, in cases with the knobs weakly infuscated. Legs with the fore coxæ darkened, the remaining coxæ and all trochanters yellow; femora yellow, the tips blackened; tibiæ and basitarsi similar, the tips more narrowly blackened; remainder of tarsi black; legs conspicuously hairy. Wings (Plate 1, fig. 12) grayish, the stigma and vague seams at origin of Rs, along cord and outer end of cell 1st M_2 slightly darker; veins light brown. Venation: Sc, ending shortly before fork of Rs, Sc_2 some distance from its tip; R_2 subequal to R_{1+2}; R_{2+3} approximately one-half to two-thirds R_3 alone; cell M_1 lacking; m straight, transverse, shorter than the arcuated basal section of M_3; m-cu close to or before midlength of cell 1st M_2.

Abdomen black, including the hypopygium, the surface more or less pruinose. Male hypopygium (Plate 3, fig. 38) with the inner dististyle, *id*, dilated at base, the outer surface with numerous erect setæ, the apex suddenly narrowed into a blackened point. Gonapophyses, *g*, appearing as slender blackened plates, the outer margin conspicuously serrate. Ædeagus, *a*, narrow, gently curved.

Habitat.—Formosa.

Holotype, male, Hassensan, altitude 6,500 to 7,800 feet, October 24, 1929 (*S. Issiki*). Allotopotype, female. Paratopotypes, 16 males and females, altitude 3,500 to 8,000 feet, October 22 to 24, 1929 (*S. Issiki*).

Limnophila (*Prionolabis*) *serridentata* is obviously closely allied to *L.* (*P.*) *liponeura* Alexander and *L.* (*P.*) *lipophleps* Alexander, of Kiushiu, Japan, differing most evidently in the structure of the male hypopygium. The somewhat similar *L. nigronitida* Edwards, likewise from the high mountains of Formosa, differs in the polished black thoracic notum and in a number of important venational characters, as the position of Sc_2, the subequal R_{2+3}, and the long oblique m.

ERIOPTERINI

TRENTEPOHLIA (MONGOMA) MONTINA sp. nov.

General coloration dark brown; legs black, the tips of the tibiæ and the tarsi paling to yellow; wings tinged with dusky; R_2 shortly before fork of R_{3+4}; fusion of Cu_1 and 1st A slight.

Male.—Length, about 6 millimeters; wing, 6.5.

Female.—Length, about 7 millimeters; wing, 6.4.

Rostrum and palpi dark brown. Antennæ black; flagellar segments with verticils of moderate length. Head black, very sparsely pruinose.

Mesonotum dark brown, the posterior margin of the scutellum and posterior half of the postnotal mediotergite more yellowish. Pleura yellowish brown, the dorsopleural region more blackish. Halteres brownish black, the base of the stem restrictedly pale. Legs with the fore coxæ dark brown, the remaining coxæ more yellowish brown; trochanters obscure yellow; femora black; tibiæ black, the tips paling to dirty yellow; tarsi yellow. Wings (Plate 1, fig. 13) with a strong dusky tinge, the stigma darker but small and ill-defined; veins brownish black. Venation: R_2 shortly before fork of R_{3+4}; m-cu at or just before the fork of M; fusion of Cu, and 1st A slight.

Abdomen brownish black, the sternites paler, especially in the male.

Habitat.—Formosa.

Holotype, male, Hassensan, altitude 4,500 to 6,000 feet, August 30, 1929 (*S. Issiki*). Allotopotype, female.

It is probable that the present species will be found to be a characteristic mountain form. It was associated with typical Palæarctic crane flies, as *Tricyphona formosana* Alexander and *Dicranota* (*Amalopina*) *gibbera* (Alexander), var.

GONOMYIA (PROGONOMYIA) CONFLUENTA (Alexander).

Gnophomyia confluenta ALEXANDER, Ann. Ent. Soc. America 17 (1924) 69.

Two males from Hassensan, altitude 3,500 to 5,500 feet, October 22, 1929 (*S. Issiki*). The venation (Plate 1, fig. 14) shows a long Sc, Sc_1 ending opposite the fork of Rs or nearly so, Sc_2 at near middistance between origin of Rs and tip of Sc_1; cell R_3 relatively deep; m-cu at or close to the fork of M.

The male hypopygium (Plate 3, fig. 39) has the outer lobe of the basistyle, *b*, stout. Three dististyles, the outer a slender curved rod from an enlarged base; second dististyle bifid at

apex, the stem with erect conspicuous setæ; inner style simple, stouter than the first, the apex obtuse. Ædeagus, *a*, compressed.

The species is very different from *G.* (*P.*) *scutellum-album* Alexander, likewise from the Formosan mountains, in the uniformly black coloration and very different male hypopygium.

GONOMYIA (GONOMYIA) NANSEI sp. nov.

General coloration dark brown; rostrum and antennæ black; head dark gray; pleura yellow, more or less distinctly variegated with brown; halteres dusky; legs brown; wings gray, the stigmal region more infuscated; male hypopygium with the outer dististyle a slender setiferous rod; phallosome with three spinous points.

Male.—Length, about 3.5 to 4 millimeters; wing, 4.5 to 5.

Female.—Length, about 4.5 to 4.8 millimeters; wing, 5 to 5.4.

Rostrum and palpi black. Antennæ black throughout, the outer flagellar segments very slender. Head dark gray.

Pronotum and anterior lateral pretergites light sulphur yellow. Mesonotum dark brown, very sparsely pruinose, the lateral and humeral regions of the præscutum yellow; scutellum light sulphur yellow; postnotal mediotergite gray. Pleura yellow, with more or less distinct darkened areas on the anepisternum and on ventral sternopleurite. Halteres elongate, dusky, the extreme base of stem yellow. Legs with the coxæ brownish testaceous; trochanters yellow; remainder of legs pale brown, the outer tarsal segments deepening to black. Wings (Plate 1, fig. 15) gray, the stigmal region more infuscated; veins darker brown. Venation: Sc_1 ending just beyond origin of Rs, Sc_2 close to its tip; cell 1st M_2 closed; m-cu at or close to fork of M.

Abdominal tergites brownish black, the sternites more yellowish. Male hypopygium (Plate 3, fig. 40) with the outer dististyle a slender setiferous rod. Inner dististyle, *id*, a flattened chitinized plate, the outer lateral angle produced into a curved blackened spine, the inner lateral angle less produced; mesal margin produced into a fleshy setiferous lobe. Phallosome, *p*, with three blackened spines, two apparently borne by an apophysal structure, the third at apex of ædeagus.

Habitat.—Formosa.

Holotype, male, Hassensan, altitude 5,600 feet, October 22, 1929 (*S. Issiki*). Allotopotype, female. Paratopotypes, 1 male, altitude 3,600 feet, August 29, 1929; 4 males and females, altitude 2,500 to 5,600 feet, October 21 to 25, 1929 (*S. Issiki*).

The specific name, *nansei*, is that of a local Formosan tribe. The fly is distinguished from all similar regional species by the structure of the male hypopygium.

GONOMYIA (LIPOPHLEPS) SAUTERI sp. nov.

General coloration dark brown; rostrum, palpi, and antennæ black; head gray; pleura dark, with a silvery white longitudinal stripe; halteres dusky; wings unmarked except for a diffuse stigma; Sc long; male hypopygium with two dististyles, the outer terminating in an acute blackened point.

Male.—Length, about 3.3 millimeters; wing, 4.1.

Rostrum and palpi brownish black. Antennæ black throughout; flagellar segments elongate, clothed with a long erect white pubescence, in addition to the longer unilaterally arranged verticils. Head dark gray, with a small yellow area on the posterior vertex.

Pronotum and anterior lateral pretergites light sulphur yellow. Mesonotal præscutum and scutal lobes uniformly dark grayish brown; median region of scutum and the scutellum except for a basal darkening obscure yellow; postnotum dark, heavily pruinose, the anterolateral portions more yellowish. Pleura dark, with a broad silvery white longitudinal stripe, extending from and including the fore coxæ, reaching the abdomen; pleurotergite chiefly yellow. Halteres dusky, including the knobs. Legs with the fore coxæ as described, the other coxæ darker, their tips pale; trochanters testaceous yellow; femora brownish yellow, passing into darker brown at tips; tibiæ and tarsi uniform brown. Wings (Plate 1, fig. 16) with a brownish tinge, the prearcular and costal regions more yellowish; the very diffuse stigma a little darker than the ground color; veins brown. Venation: Sc long, Sc_1 extending to shortly before midlength of the long Rs, Sc_2 some distance from its tip; branches of Rs strongly divergent; R_5 and M_{1+2} deflected toward one another at margin, narrowing the cell; cell 1st M_2 narrowed at base.

Abdominal tergites uniformly dark brown, the hypopygium more yellowish. Male hypopygium (Plate 3, fig. 41) with two dististyles, the outer, *od*, a slender rod that gradually narrows to an acute blackened point, the base of the latter with numerous setæ. Inner dististyle a small pale lobe that is a little shorter than the apical lobe of the basistyle, terminating in a fasciculate bristle and with a very long slender seta on outer margin beyond

midlength. Phallosome, *p*, appearing as a broad pale plate, the surface laterally with abundant microscopic setulæ, the tip produced into a glabrous portion; two additional elongate rods, the shorter from an enlarged base.

Habitat.—Formosa.

Holotype, male, Daitotei, April 1914 (*H. Sauter*).

Type in the collection of the Deutsches Entomologisches Museum.

Gonomyia (Lipophleps) sauteri is named in honor of Mr. H. Sauter, well-known collector of Formosan insects. The species is very distinct from *G. (L.) longiradialis* Alexander (Luzon) and *G. (L.) skusei* Alexander (eastern Australia) in the structure of the male hypopygium. This is very probably the species recorded as *skusei* (*gracilis* Skuse, preoccupied) by Riedel[1] from Macuyama, Formosa.

GONOMYIA (LIPOPHLEPS) NEONEBULOSA sp. nov.

General coloration dark brownish gray; pleura dark, with a yellowish white longitudinal stripe; femora brownish black; wings gray, variegated with brownish gray; costal region pale yellow; abdomen grayish black, the caudal margins of the segments narrowly yellow.

Female.—Length, about 4.5 millimeters; wing, 4.1.

Rostrum and palpi black. Antennæ with the scapal segments obscure orange; flagellum black. Head orange, the disk of the vertex darkened.

Pronotum and anterior lateral pretergites light yellow, the former darkened laterally. Mesonotum dark brownish gray, the caudal margin of the scutellum broadly yellow; postnotal mediotergite pale, with a little less than the distal half darkened. Pleura dark, with a broad, yellowish white, longitudinal stripe extending from and including the fore coxæ, passing beneath the halteres to the abdomen; dorsal pleurites brown, the ventral sclerites more bluish gray. Halteres with the stem dusky at base, the distal half yellow; knobs dusky, the ends conspicuously yellow. Legs with the fore coxæ yellow; remaining coxæ similar, the bases narrowly darkened; femora brownish black beyond the base, the extreme tips on lower surface a little brightened; tibiæ and tarsi brownish black. Wings (Plate 1, fig. 17) with the ground color gray, variegated with darker brownish gray clouds; cells C and Sc pale yellow; stigma not darker than the

[1] Archiv für Naturgeschichte 82 for 1916, Abteil. A (1917) 112.

remaining dark areas; darkened clouds occupying most of radial field, except an area in outer end of cell R; other fields of wing merely streaked with dark; veins brown, Sc yellow. Venation: Sc_1 ending some distance before origin of Rs, the latter vein sinuous at end; anterior branch of Rs relatively long and straight, cell R_2 being nearly parallel; m-cu close to fork of M.

Abdomen grayish black, the caudal margins of the segments narrowly yellow.

Habitat.—Formosa.

Holotype, female, Meizi Hot Springs, foot of Hassensan, altitude 2,500 feet, October 25, 1929 (*S. Issiki*). Paratopotype, female; paratype, 1 sex?, Hassensan, altitude 3,600 feet, August 29, 1929 (*S. Issiki*).

By Edwards's key to the Oriental species of *Lipophleps* * the present species runs to *G.* (*L.*) *robinsoni* Edwards, of the Federated Malay States, differing in slight details of coloration and venation.

GONOMYIA (LIPOPHLEPS) SINUOSA sp. nov.

General coloration brown; basal segments of antennæ orange; head yellow, the center of the vertex blackened; a conspicuous silvery longitudinal stripe on pleura; legs brownish yellow, the tarsi passing into black; wings brownish yellow, the stigmal and axillary regions a little darkened; anterior branch of Rs sinuous on distal third.

Female.—Length, about 5 millimeters; wing, 4.5.

Rostrum and palpi black. Antennæ with scapal segments orange, the flagellum black. Head yellow, the center of the vertex blackened.

Pronotum and anterior lateral pretergites light sulphur yellow, the former dark brown on sides. Mesonotum light brown, slightly darker medially; scutal lobes brown, the median region yellow; scutellum brown, narrowly margined caudally with paler; postnotum pale, sparsely pruinose. Pleura light brown, with a conspicuous silvery white longitudinal stripe extending from behind the fore coxæ to the base of the abdomen, this passing beneath the halteres; the stripe is margined both above and below by a narrower infuscated line; ventral sternopleurite and dorsal pleurites, including the pleurotergite, more buffy yellow. Halteres yellow, the knobs a little more obscure. Legs with the coxæ and trochanters yellow; femora obscure brownish yellow,

* Journ. Fed. Malay St. Mus. 14 (1928) 104-105.

the tibiæ gradually darkening; tarsi passing into black. Wings (Plate 1, fig. 18) with a faint brownish yellow tinge; costal region clearer yellow; stigma appearing as a longitudinal dusky streak in cell R_2, the remainder of the region yellow; axillary region a little darkened; outer ends of the radial cells slightly darkened; veins pale brown, Sc yellower. Venation: Sc short, Sc, ending some distance before the origin of Rs, the latter angulated at origin; anterior branch of Rs sinuous, the distal third arcuate and evidently marking the point of departure of a small cephalic branch, R_3, normally present in the *sulphurella* group; m-cu shortly before the fork of M.

Abdominal tergites brown, the segments narrowly margined caudally with pale yellow; sternites more yellowish, the caudal margins narrowly paler yellow, the lateral margins narrowly infuscated, most distinct on the basal segments.

Habitat.—Formosa.

Holotype, female, Meizi Hot Springs, foot of Hassensan, altitude 2,500 feet, October 25, 1929 (*S. Issiki*).

Gonomyia (*Lipophleps*) *sinuosa* is well distinguished from other regional species by the details of coloration and venation. The course of the anterior branch of Rs is very peculiar and suggestive.

CRYPTOLABIS (BAEOURA) TRICHOPODA sp. nov.

General coloration black; head gray; halteres with yellow knobs; legs brown, conspicuously hairy; wings strongly tinged with blackish, streaked longitudinally with pale; cell 2d A narrow; male hypopygium with the dististyle a simple curved blade, the tip acute.

Male.—Length, about 3.8 millimeters; wing, 4.5.

Rostrum and palpi dark brown. Antennæ black throughout; second scapal segment oval; flagellar segments gradually decreasing in size outwardly; verticils elongate. Head dull gray, the orbits paler.

Pronotum black. Mesonotum black, the very restricted anterior and posterior lateral pretergites, together with the humeral region of the præscutum yellow; scutellum orange, the base blackened medially. Pleura black. Halteres black, the knobs and extreme base of stem yellow. Legs with the coxæ black; trochanters brownish yellow; femora and tibiæ brown, the tips darker; tarsi more blackened; segments of legs with very long conspicuous erect setæ. Wings (Plate 1, fig. 19)

strongly tinged with blackish, the elongate stigmal region slightly darker; pale longitudinal streaks in costal region, and along veins M, M_{3+4}, and 1st A; veins dark brown. Macrotrichia of veins long and conspicuous. Venation: Sc_1 ending opposite fork of Rs, Sc_2 some distance from tip of Sc_1; cell 2d A narrow.

Abdomen dark brown, the hypopygium blackened, the dististyles yellow. Male hypopygium (Plate 3, fig. 42) with the dististyle, *d*, a curved blade that terminates in an acute point, the distal half with numerous small setæ. What may be a tergal structure appears as two pale truncated cushions, densely set with short setulæ, each lobe with a single longer bristle. There is a possibility that these structures are really gonapophyses, but if so they are very different from those of other allied species.

Habitat.—Formosa (south).

Holotype, male, Keinensan, near Heito, altitude 5,000 feet, March 13, 1929 (*S. Issiki*).

Cryptolabis trichopoda is very distinct from *C. aliena* (Alexander), the only other Formosan species of the genus. In general appearance it is more like the Indian *C. funebris* (Alexander), differing conspicuously in the structure of the dististyles of the male hypopygium.

ERIOPTERA (EMPEDA) SULFUREOCLAVATA sp. nov.

General coloration gray; scapal segments of antennæ black; legs light brown; halteres with conspicuous sulphur yellow knobs; wings gray, the stigmal region scarcely darker; Sc long; anal veins strongly divergent; male hypopygium with the dististyles unblackened.

Male.—Length, about 2.8 millimeters; wing, 3.5.

Rostrum gray; palpi black. Antennæ with the scapal segments black, the flagellum pale brownish yellow, the outer segments darker. Head light gray.

Mesonotum dark brownish gray, the lateral pretergites light sulphur yellow, the posterior sclerites of notum clearer gray. Pleura dark gray, the dorsopleural region pale yellowish white. Halteres pale, the knobs light sulphur yellow. Legs with the fore coxæ dark brown, the remaining coxæ and trochanters brownish yellow; remainder of legs light brown, the terminal tarsal segments darker. Wings (Plate 1, fig. 20) broad, iridescent gray, the prearcular and costal portions more yellowish;

stigma scarcely darker; veins pale brown, those in the costal region more yellowish. Macrotrichia of veins relatively short. Venation: Sc long, Sc_1 ending beyond midlength of Rs, Sc_2 a short distance from its tip; R_3 of moderate length, longer than R_{3+4}; m-cu at fork of M; vein 2d A short and nearly straight, the anal veins strongly divergent; cell 1st A very wide at margin.

Abdomen dark brown, the incisures narrowly pale; hypopygium yellow. Male hypopygium (Plate 3, fig. 43) with the outer lobe of basistyle, *b*, terminating in two or three very long setæ; inner lobe stouter, densely set with erect setæ. Dististyles both broad, not blackened, the outer style bifid.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 4,500 feet, August 14, 1929 (*ex Parish*).

Erioptera (*Empeda*) *sulfureoclavata* is readily separated from all regional species with long subcosta by the coloration, as the conspicuous sulphur yellow knobs of the halteres, and the venation, as the length and course of vein R, and the strong divergence of the anal veins.

ERIOPTERA (EMPEDA) LILIPUTINA sp. nov.

Size very small (wing, male, 2.6 millimeters); general coloration grayish black; halteres dusky, the knobs obscure yellow; legs black; wings strongly suffused with blackish; Sc long; cell 1st M_2 closed.

Male.—Length, about 2 millimeters; wing, 2.6.

Rostrum and palpi brownish black. Antennæ black throughout. Head dark brownish gray.

Pronotum brownish black. Anterior lateral pretergites very restrictedly obscure yellow. Mesonotum dull grayish black, the pseudosutural foveæ and tuberculate pits more intense black; scutellum somewhat more pruinose. Pleura grayish black. Halteres dusky, the knobs obscure yellow. Legs with the coxæ blackish; trochanters brownish yellow; remainder of legs brownish black. Wings (Plate 1, fig. 21) with a strong blackish suffusion, the veins darker. Venation: Sc long, Sc_1 extending to beyond one-third the length of Rs, Sc_2 a short distance from its tip; Rs relatively long and straight; vein R_3 straight, the cell thus pointed at base; cell 1st M_2 closed; m-cu erect, placed just beyond the fork of M.

Abdomen black, including the hypopygium. Male hypopygium with the outer dististyle blackened, the dististyle bifid, the outer arm densely covered with microscopic setulæ. Inner

dististyle a straight slender rod, the apex obtuse. Gonapophyses appearing as pale flattened blades.

Habitat.—Formosa.

Holotype, male, Hassensan, altitude 3,600 feet, August 29, 1929 (*S. Issiki*).

Erioptera (*Empeda*) *liliputina* is readily told from all described regional species by the small size and venation, notably the closed cell 1st M_2.

ORMOSIA ANTHRACOPODA sp. nov.

General coloration gray; halteres yellow; legs black; wings cream-colored, with a sparse brown pattern; male hypopygium with the apex of the basistyle produced into a small spine.

Male.—Length, about 6 millimeters; wing, 7.

Rostrum and palpi black. Antennæ black throughout, of moderate length; verticils of the basal flagellar segments long, becoming shorter on the outer segments. Head gray.

Mesonotal præscutum light gray with four darker brownish gray stripes that are inconspicuous against the ground color; pseudosutural foveæ and tuberculate pits black; scutal lobes brownish gray; scutellum and postnotum clearer gray. Pleura clear gray. Halteres yellow. Legs with the coxæ and trochanters brownish yellow; remainder of legs black. Wings (Plate 1, fig. 22) cream-colored, with a slight brownish tinge; stigma brown; restricted darker brown clouds at origin of Rs, Sc_2, along cord, m, and tips of most longitudinal veins; relatively inconspicuous whitish areas before and beyond the stigma; veins brown, darker in the clouded areas. Venation: Sc, ending opposit R_2; Sc_2 about opposite one-fifth the length of Rs; R_2 just beyond the fork of R_{2+3+4}; tips of veins R_3 and R_4 deflected cephalad; point of union of distal section of M_3 with m angulated and weakly spurred; m-cu shortly before fork of M; vein 2d A sinuous on distal half.

Abdomen brownish gray, the hypopygium dark. Male hypopygium (Plate 3, fig. 44) with the tip of the basistyle, *b*, produced into a small acute spine. Two dististyles, one a strongly curved sickle-shaped spine, extended into a long straight blackened point, the margin with very long erect setæ; second dististyle nearly straight, the basal half more dilated. Gonapophyses small, in general outline nearly like the inner dististyle, the apical spine more acute.

Habitat.—Formosa.

Holotype, male, Hassensan, altitude 6,500 to 7,800 feet, October 26, 1929 (*S. Issiki*).

Ormosia anthracopoda is very distinct from the other species of the genus described from Formosa. It belongs to the *aculeata* group, having the apex of the basistyle of the male hypopygium produced into an acute spine.

STYRINGOMYIA TAIWANENSIS sp. nov.

General coloration pale yellow; legs uniformly yellow or with scarcely indicated pale brown bands on femora; wings yellow, the veins along cord and outer ends of cell 1st M_2 a little darker; vein 2d A gently curved to the margin; male hypopygium with three apical spines on basistyle, one more isolated and terminating in an obtuse knob that is further abruptly narrowed into a hairlike spine.

Male.—Length, about 7 millimeters; wing, 5 to 5.5.

Female.—Length, about 5 to 5.5 millimeters; wing, 5 to 5.4.

Rostrum yellow; palpi alternately yellow and dark brown. Antennal flagellum pale yellow, the scapal segments scarcely darker. Head light sulphur yellow.

Pronotum sulphur yellow. Mesonotum more testaceous yellow, without dark markings. Pleura testaceous yellow. Halteres pale, the knobs light yellow. Legs uniformly yellow, in cases the femora with scarcely indicated pale brown bands; terminal tarsal segments black. Wings (Plate 1, fig. 23) yellow, with a sparse brown clouding along the cord and outer end of cell 1st M_2, most evident on r-m; veins yellow, darker in the infuscated areas. Venation: Vein 2d A curved gently to the margin.

Abdomen yellow, the caudal margins of the intermediate tergites narrowly dark brown. Male hypopygium (Plate 3, fig. 45) with the ninth sternite, 9*s*, broad, the apical spines widely separated. Basistyle terminating in three conspicuous spines, two being long and extended into acute points, the third arising from a separate apical lobe of the basistyle, knobbed at apex, thence further produced into a hairlike point. Dististyle, *d*, complicated in structure, the long slender outer arm bearing a very elongate subterminal seta; margin of arm near base with a series of peglike spines. Spines of intermediate arms of dististyle unusually long and slender.

Habitat.—Formosa.

Holotype, male, Hassensan, altitude 3,500 feet, October 21, 1929 (*S. Issiki*). Allotopotype, female, in copula with the type.

Paratopotypes, 6 males and females, altitude 2,500 to 5,500 feet, October 22 to 25, 1929 (*S. Issiki*); paratype, 1 female, Funkiko, April 21, 1917 (*T. Shiraki*).

Styringomyia taiwanensis has been confused with *flava* Brunetti and the paratype was earlier recorded as being that species.[1] The present form differs in the coloration of the wings and details of structure of the male hypopygium.

STYRINGOMYIA SINENSIS sp. nov.

General coloration yellow, the mesonotum largely black, more or less variegated with pale at the suture; halteres yellow; femora and tibiæ with narrow brownish black rings; wings yellow, with a sparse dark pattern, including the vicinity of r-m, outer end of cell 1st M_2 and end of vein 2d A; small dark marginal spots at ends of medial and cubital veins; vein 2d A strongly angulated and weakly spurred at margin; male hypopygium with the dististyle very large and complex, four-lobed, the two intermediate lobes with combs of spines.

Male.—Length, about 7 to 8 millimeters; wing, 5.2 to 6.

Female.—Length, about 6 to 6.5 millimeters; wing, 4.6 to 5.

Antennæ with the scape black; flagellum yellow. Head chiefly ocherous; a dark spot touching inner margin of eye at narrowest point of vertex.

Mesonotum extensively to almost entirely blackened, more or less pruinose, in certain of the specimens brightened at the suture; the amount of pale coloring variable, in some specimens involving the posterior third of the præscutum and the scutal lobes, the anterior portion of præscutum always blackened. Pleura abruptly and uniformly yellow. Halteres pale, the knobs bright yellow. Legs with the coxæ and trochanters yellow; femora yellow, with two narrow dark rings that are interrupted beneath; tibiæ yellow, the tips narrowly blackened, with a narrower ring at just before midlength, this obsolete on lower surface; tarsi yellow, the tips of tarsal segments one and two darkened; terminal segment dark brown. Wings (Plate 1, fig. 24) yellow, the veins a little darker yellow; a restricted dark pattern, arranged as follows: At r-m, involving the ends of all surrounding veins; outer end of cell 1st M_2; on m-cu and its junction with Cu_1; tip of 2d A; small darkened marginal areas at ends of veins R_5 to Cu_{1a}, inclusive. Venation: 2d A angularly

[1] Ann. Ent. Soc. America 13 (1920) 253.

bent into the margin, this curvature rectangular or acute, usually with a short spur at the bend.

Abdomen yellow, the tergites with geminate brown spots at caudal margin, with vague indications of paler brown markings on the basal ring; on segment seven with a median brown stripe; hypopygium pale. Male hypopygium (Plate 3, fig. 46) with the dististyle, *d*, four-lobed, the outer lobe long and slender, bearing the usual very long apical seta; second arm bilobed at apex, the margin with groups of long black spines to produce a comblike appearance; third lobe flattened, with a ⌒-shaped series of shorter peglike spines; innermost lobe bearing an apical series of very long setoid spines. Ninth sternite, 9*s*, slender, with two apical setæ that are placed close together. Ninth tergite without lateral shoulders, as found in *mahensis*.

Habitat.—Western China and Formosa.

Holotype, male, Mount Omei, Szechwan, China, altitude 4,500 feet, August 4, 1929 (*ex Parish*). Allotopotype, female. Paratopotypes, 15 males and females, August 2 to 19, 1929; paratypes, 8 males and females, Hassensan, Formosa, altitude 2,500 to 3,500 feet, October 21 to 25, 1929 (*S. Issiki*).

By Edwards's key to the species of *Styringomyia*[1] the present fly runs to *S. mahensis* Edwards, an otherwise very different fly. The structure of the male hypopygium separates *S. sinensis* from all regional forms.

[1] Trans. Ent. Soc. London (1914) 210–212.

ILLUSTRATIONS

(Legend: *a*, aedeagus; *b*, basistyle; *d*, dististyle; *g*, gonapophysis; *id*, inner dististyle; *od*, outer dististyle; *p*, phallosome; *s*, sternite; *t*, tergite; *vd*, ventral dististyle.)

PLATE 1

FIG. 1. *Limonia* (*Discobola*) *taivanella* sp. nov., venation.
2. *Limonia* (*Limonia*) *koxinga* sp. nov., venation.
3. *Limonia* (*Libnotes*) *hassenana* sp. nov., venation.
4. *Limonia* (*Rhipidia*) *triarmata* sp. nov., venation.
5. *Limonia* (*Dicranomyia*) *subpunctulata* sp. nov., venation.
6. *Limonia* (*Geranomyia*) *apicifasciata* sp. nov., venation.
7. *Antocha* (*Antocha*) *styx* sp. nov., venation.
8. *Helius* (*Eurhamphidia*) *perelegans* sp. nov., venation.
9. *Dicranoptycha issikina* sp. nov., venation.
10. *Dicranota* (*Amalopina*) *delectata* sp. nov., venation.
11. *Adelphomyia issikina* sp. nov., venation.
12. *Limnophila* (*Prionolabis*) *serridentata* sp. nov., venation.
13. *Trentepohlia* (*Mongoma*) *montina* sp. nov., venation.
14. *Gonomyia* (*Progonomyia*) *confluenta* Alexander, venation.
15. *Gonomyia* (*Gonomyia*) *nansei* sp. nov., venation.
16. *Gonomyia* (*Lipophleps*) *sauteri* sp. nov., venation.
17. *Gonomyia* (*Lipophleps*) *neonebulosa* sp. nov., venation.
18. *Gonomyia* (*Lipophleps*) *sinuosa* sp. nov., venation.
19. *Cryptolabis* (*Baeoura*) *trichopoda* sp. nov., venation.
20. *Erioptera* (*Empeda*) *sulfureoclavata* sp. nov., venation.
21. *Erioptera* (*Empeda*) *liliputina* sp. nov., venation.
22. *Ormosia anthracopoda* sp. nov., venation.
23. *Styringomyia taiwanensis* sp. nov., venation.
24. *Styringomyia sinensis* sp. nov., venation.

PLATE 2

FIG. 25. *Dolichopeza* (*Nesopeza*) *tarsalba* sp. nov., male hypopygium, lateral.
26. *Dolichopeza* (*Nesopeza*) *tarsalba* sp. nov., male hypopygium, ninth tergite.
27. *Dolichopeza* (*Nesopeza*) *tarsalba* sp. nov., male hypopygium, gonapophysis.
28. *Dolichopeza* (*Nesopeza*) *tarsalba* sp. nov., male hypopygium, eighth sternite.
29. *Dolichopeza* (*Oropeza*) *saitamensis* sp. nov., male hypopygium, ninth tergite.
30. *Dolichopeza* (*Oropeza*) *saitamensis* sp. nov., male hypopygium, inner dististyle.

FIG. 31. *Limonia* (*Discobola*) *taiwanella* sp. nov., male hypopygium.
32. *Limonia* (*Limonia*) *koxinga* sp. nov., male hypopygium.
33. *Limonia* (*Rhipidia*) *triarmata* sp. nov., male hypopygium.
34. *Limonia* (*Dicranomyia*) *subpunctulata* sp. nov., male hypopygium.
35. *Limonia* (*Geranomyia*) *apicifasciata* sp. nov., male hypopygium.
36. *Antocha* (*Antocha*) *styx* sp. nov., male hypopygium.
37. *Dicranoptycha issikina* sp. nov., male hypopygium.

PLATE 3

FIG. 38. *Limnophila* (*Prionolabis*) *serridentata* sp. nov., male hypopygium.
39. *Gonomyia* (*Progonomyia*) *confluenta* (Alexander), male hypopygium.
40. *Gonomyia* (*Gonomyia*) *nansei* sp. nov., male hypopygium.
41. *Gonomyia* (*Lipophleps*) *sauteri* sp. nov., male hypopygium.
42. *Cryptolabis* (*Baeoura*) *trichopoda* sp. nov., male hypopygium.
43. *Erioptera* (*Empeda*) *sulfureoclavata* sp. nov., male hypopygium.
44. *Ormosia anthracopoda* sp. nov., male hypopygium.
45. *Styringomyia taiwanensis* sp. nov., male hypopygium.
46. *Styringomyia sinensis* sp. nov., male hypopygium.

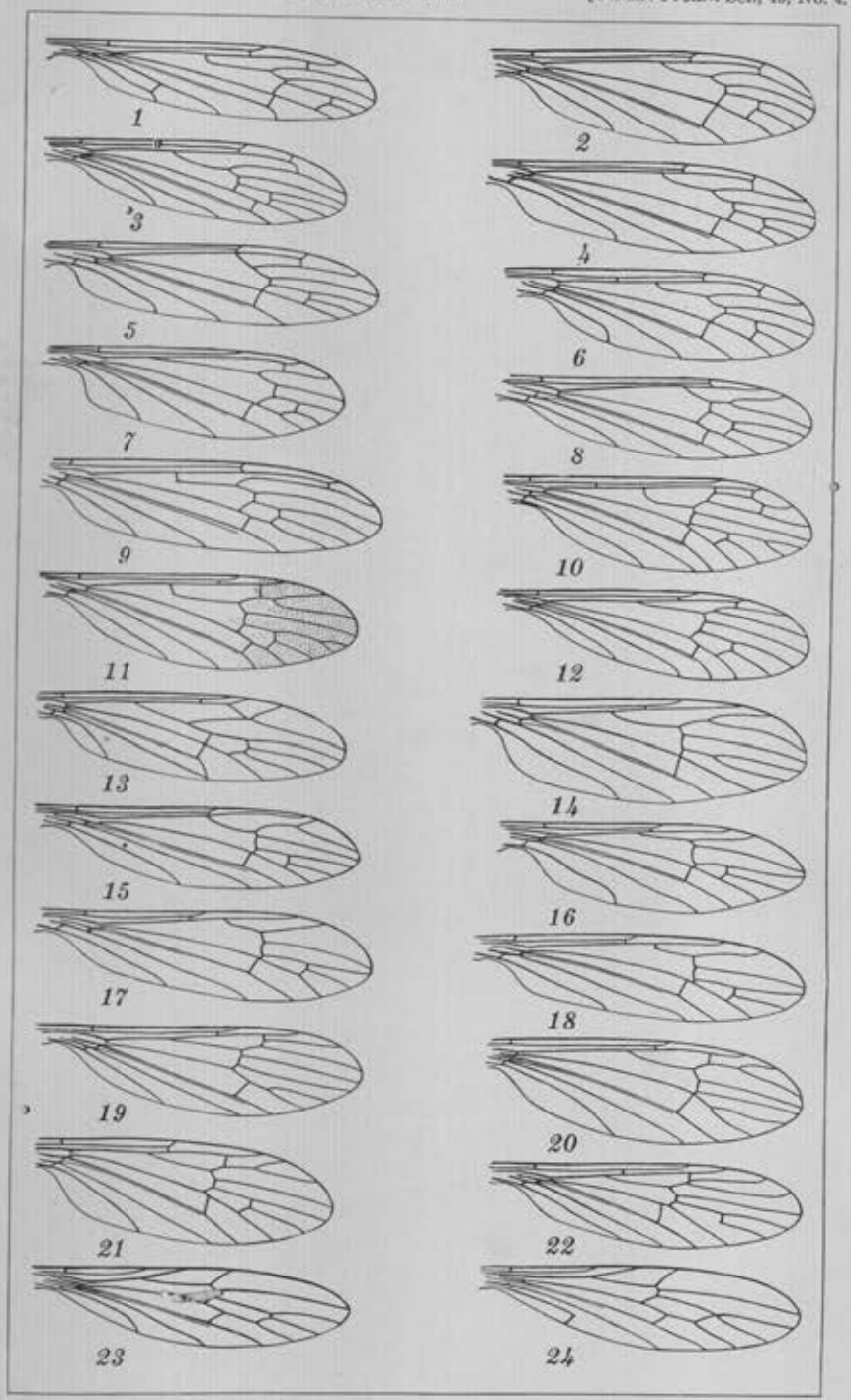

PLATE 1.

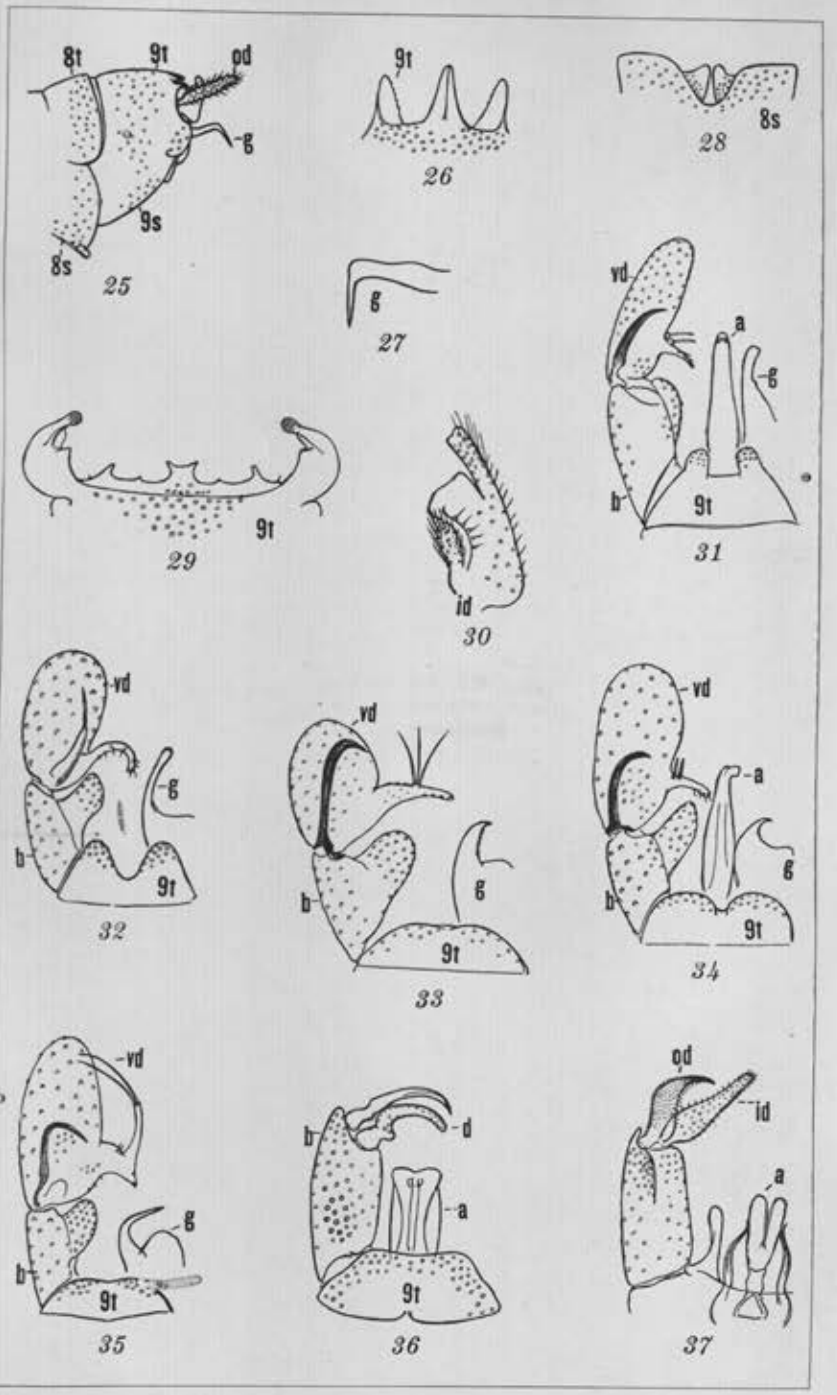
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PLATE 2.

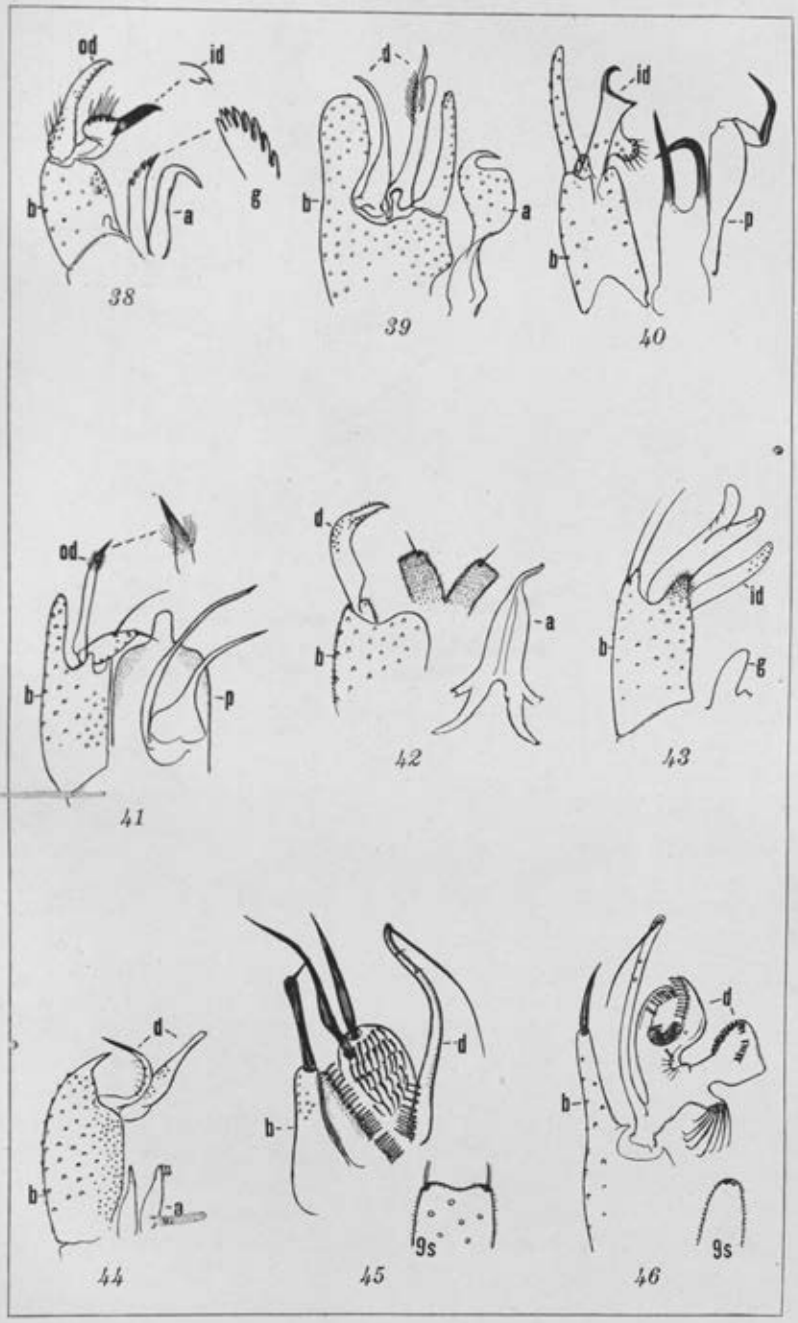
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PLATE 3.

SIXTH REPORT UPON DIPTERA PUPIPARA FROM THE PHILIPPINE ISLANDS

By G. F. Ferris

Of Stanford University, California

SEVEN TEXT FIGURES

For the material upon which this sixth report is based, I am as before indebted chiefly to Mr. R. C. McGregor. It has been my desire to present in these papers something approaching a review of the genera of the Hippoboscidæ, at least as far as this family is represented in the Philippine Islands. Because of this, there is a departure in the present report from the procedure followed in the earlier papers of the series. Certain species that are not at present known from the Philippine Islands, but which may reasonably be assumed to occur there, are here included. That these species will eventually be collected in the Islands is certain, and their inclusion herein permits at least some discussion of probably all the genera of the Hippoboscidæ that occur in the Philippines, with the exception of *Stenopteryx*. This genus is known from Europe and India, where it occurs on swallows, and is to be looked for on these birds elsewhere.

Genus HIPPOBOSCA Linnæus

This genus, the type of the Hippoboscidæ, may be characterized as follows: Hippoboscidæ with functional, noncaducous wings in which there are several veins behind the costa; with but two "cross veins" and consequently without an anal cell; with the wing membrane showing a series of slight, radiating furrows and ridges in the nonveined portion. Claws simple. Antennæ almost entirely concealed within their pits. Prothorax with a distinct, more or less sclerotic, pronotal plate, the humeral processes of the mesothorax lacking, the head consequently appearing as relatively free from the thorax and not received into its anterior border as in most members of the family. Ocelli lacking. Abdomen with the derm somewhat squamose-reticulate, but without a distinct median, dorsal area of transverse striations.

Type of the genus, *Hippobosca equina* Linnæus.

Notes.—This genus, as the type of the Hippoboscidæ, stands in need of a thorough comparative morphological study. It is not my intention to attempt such a study in connection with the present paper, but certain morphological features which are of systematic importance within the family are here dealt with on the basis of the two species *H. equina* Linnæus and *H. maculata* Leach, the former being the type of the genus. Neither of these species appears to have been recorded from the Philippine Islands, although they surely are to be found there.

The species of this genus are among the most striking members of the family, owing to the coloration of the thoracic dorsum. Very little consideration has been given by earlier arthors to the morphology of the group and specific differentiation has been based chiefly upon these colors, with the result that there are probably several more names than there are species. Six alleged species are at hand, but this is not the place for a discussion of them.

In some respects this genus appears to be rather isolated from the remainder of the family, so much so that Speiser has placed it as the sole member of the subfamily Hippoboscinæ. I am not myself so much impressed by its peculiar features and am inclined to regard it as definitely a member of the group of mammal-infesting genera including certainly *Lipoptena, Echestypus, Melophagus,* and probably *Ortholfersia* and *Allobosca.*

The palpi in *Hippobosca* (fig. 4, *b*) are well developed and are strongly deflected. The clypeal region (fig. 4, *b*) is quite deeply emarginate. The antennæ are short and almost concealed within the open antennal pits, the second segment more or less globular (fig. 4, *e*) and containing the invaginated third segment from which rises the antleriform arista; the first segment is not recognizable.

The head is somewhat less flattened than in most of the Hippoboscidæ and is without a sharp, thin, occipital epiphysis. The prothorax presents a very definite pronotal plate, in contrast to all the other Hippoboscidæ in which this region is membranous, and in *H. maculata* and similar species this bears a transverse row of setæ. The humeral processes are entirely lacking. The remainder of the thorax (fig. 3) presents no unusual features except the pronounced development of the swollen pleurotergites. The claws (fig. 4, *h*) are simple, with large, flat pulvilli and a stout, setiform empodium.

The wings (fig. 2) have as their chief peculiarity the development of a series of ridges and furrows radiating from the veins to the posterior margin. Structurally these are indicated only by more or less faint lines of pigmentation. The wings are entirely devoid of minute setulæ.

The abdomen is more or less thickly beset with setæ of various sizes, each borne upon a small, sclerotic ring. In a few forms these rings are present but in part seem to bear no setæ. The remainder of the derm is for the most part marked with a mosaic of small sclerotic or pigmented scalelike markings, but there is no such dorsal area of transverse striations as appears in the Olfersiinæ. There is the usual large tergal and small sternal basal plate. In *H. equina* there are three additional, small tergal plates and in all available material there are two pairs of large and heavily sclerotic, swollen, subapical plates, except in the male of *H. equina* which has but one such pair. There is but comparatively little sexual dimorphism. The claspers of the male are very small, and the genital and anal openings tend to be more or less strongly retracted to the ventral side of the body. In the male of *H. equina* the tergal plates are larger than in the female.

I have not attempted to work out the details of the copulatory apparatus of the male.

HIPPOBOSCA EQUINA Linnæus. Figs. 1; 2, a; 3; 4, a, b, d–h.

AUSTEN, Ann. & Mag. Nat. Hist. VII 12 (1903) 256.

MASSONAT, Ann. de l'Univ. de Lyon (nouvelle série) 1 Fasc. 28 (1909) 235–243, pl. 1, figs. 1–5.

STEKHOVEN, Die Bloedzuigende Arthropoda van Nederl. Oost Indie 1–2 (1923) 83–92; figs.

Material examined.—Several individuals from northern Africa and from Java, received through the kindness of the late Dr. M. Bezzi and Dr. J. H. Schuurmans Stekhoven.

Notes.—This species is apparently very common in Europe and northern Africa, and since it has been recorded from Burma, Java, and New Caledonia its presence in the Philippine Islands may be taken for granted, even in the absence of definite records. Its typical host is considered to be the domestic horse, although Massonat has recorded it from cattle, dromedary, dog, and even birds.

I would at this point call attention to the very close resemblance between this species and the supposedly distinct *H. capensis* von Olfers (=*H. francilloni* Leach and *H. canina*

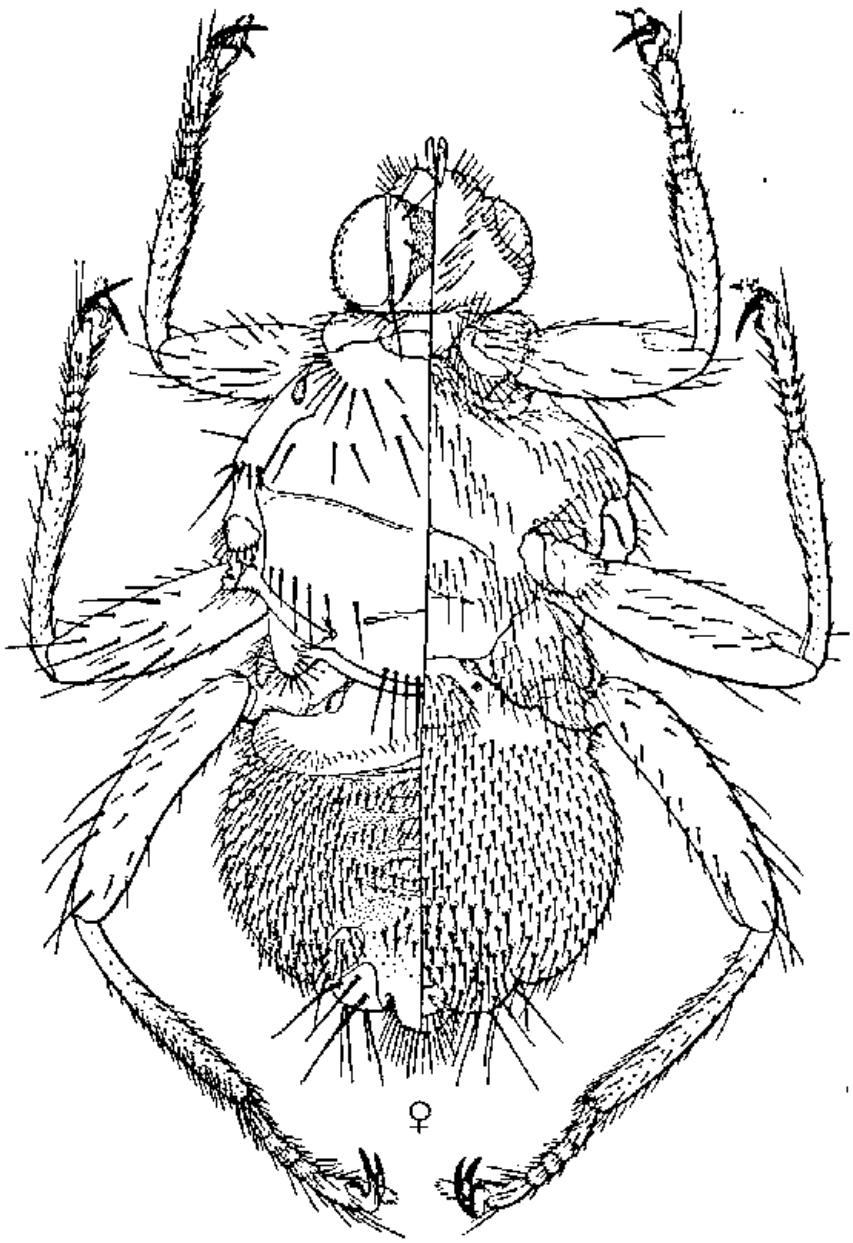

FIG. 1. *Hippobosca equina* Linnæus; female, wings removed. From a specimen from the domestic horse in Java.

Rondani), which occurs commonly on dogs in Africa and apparently almost throughout Asia. I have at hand specimens of this, determined by Bezzi as *H. capensis*, from northern Africa and other specimens from China from dogs. Austen has called attention to the fact that the color differences supposed to differentiate these two species do not hold and has indicated that they may be separated only by the color of the wing veins, typical *H. capensis* having the veins pale except for their noticeably dark apices, while *H. equina* has the veins uniformly colored.

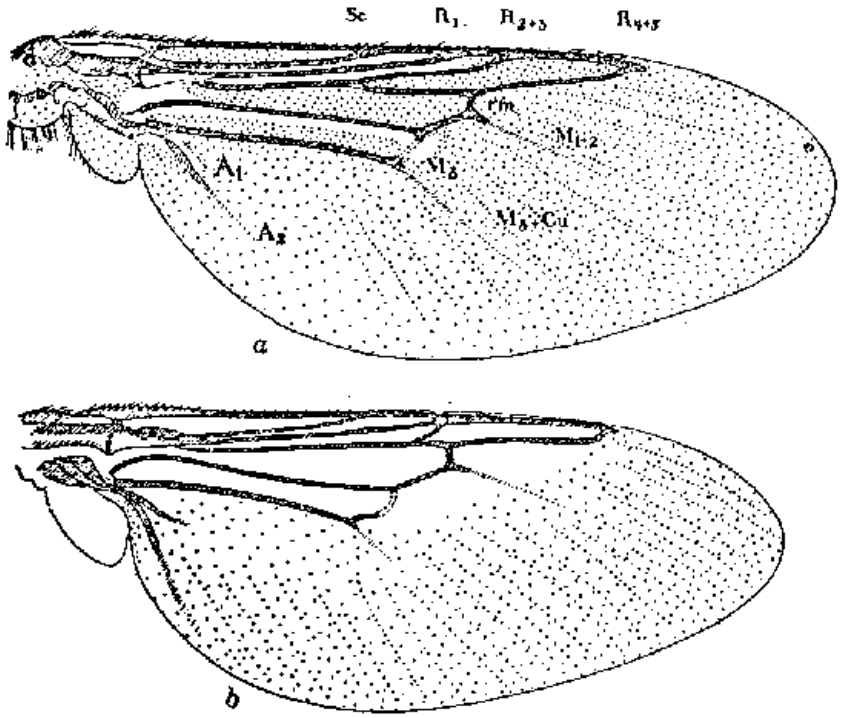

FIG 2. *Hippobosca equina* Linnæus; *a*, wing. *Hippobosca maculata* Leach; *b*, wing. The figures are drawn to slightly different scales.

Structurally there appears to be no difference whatsoever between these two supposed species. There is a considerable degree of variation in the form and size of the setæ of the abdomen, but this does not appear to correlate with the kind of host and may be regarded as individual. An examination of more material is desirable, but it is my personal belief that the two species are the same thing.

As the species is the type of its genus and, consequently, of the Hippoboscidæ, I shall here consider it at some length. The

color pattern has been described by many authors and is figured by Stekhoven and will here be passed over. The species is one of the smaller members of its genus, reaching a length of not more than 10 millimeters in the most fully expanded females, with a wing length of 6 millimeters, while *H. camelina* reaches a total length of 12 millimeters with a wing length of 8 millimeters.

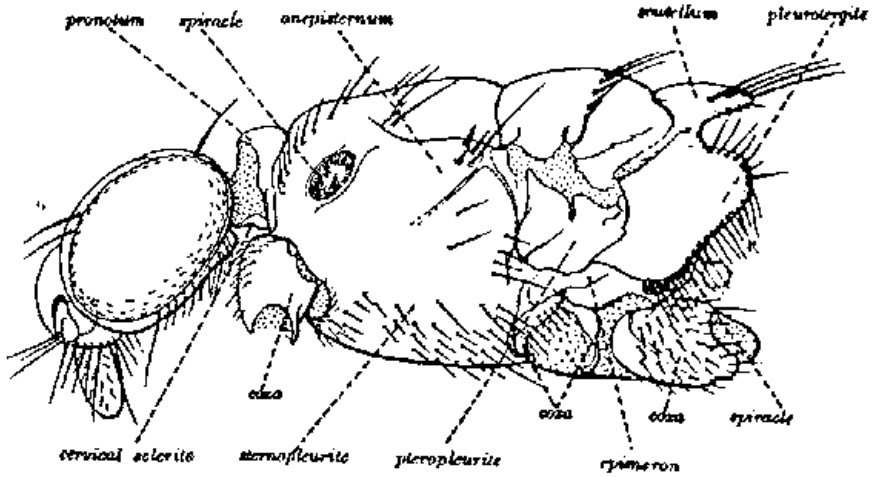

FIG. 3. *Hippobosca equina* Linnæus; lateral aspect of head and thorax.

The body (fig. 1) is rather noticeably hairy, the hairs for the most part being pale. There is a considerable actual variation in size and shape of the abdominal setæ, and an apparent variation in their distribution due to changes in the size and shape of the abdomen.

The palpi are short and broad and strongly deflexed. The clypeus (fig. 4, *b*) is distinctly emarginate. The antennæ are short, retracted into deep pits, with the first segment not distinguishable and with the second (fig. 4, *d*) more or less globular and enveloping the small third segment (fig. 4, *f*) from which rises the branched arista. The head as a whole is rather quadrate in form.

The pronotum is developed as a distinct plate, which does not, however, bear a transverse row of setæ as in *H. maculata* and some other species. The humeral processes of the mesothorax are entirely lacking. The scutellum is small and rounded and the pleurotergites quite strongly swollen. In fig. 3 is shown the lateral aspect of the thorax and head, with the parts interpreted in accord with the views of recent students of the

Diptera. The legs present nothing unusual; the claw is shown in fig. 4, *h*.

The wing (fig. 2, *a*) shows in slide preparations but little evidence of the ridges and furrows that appear in dry specimens, these being indicated only by faint lines of pigmentation.

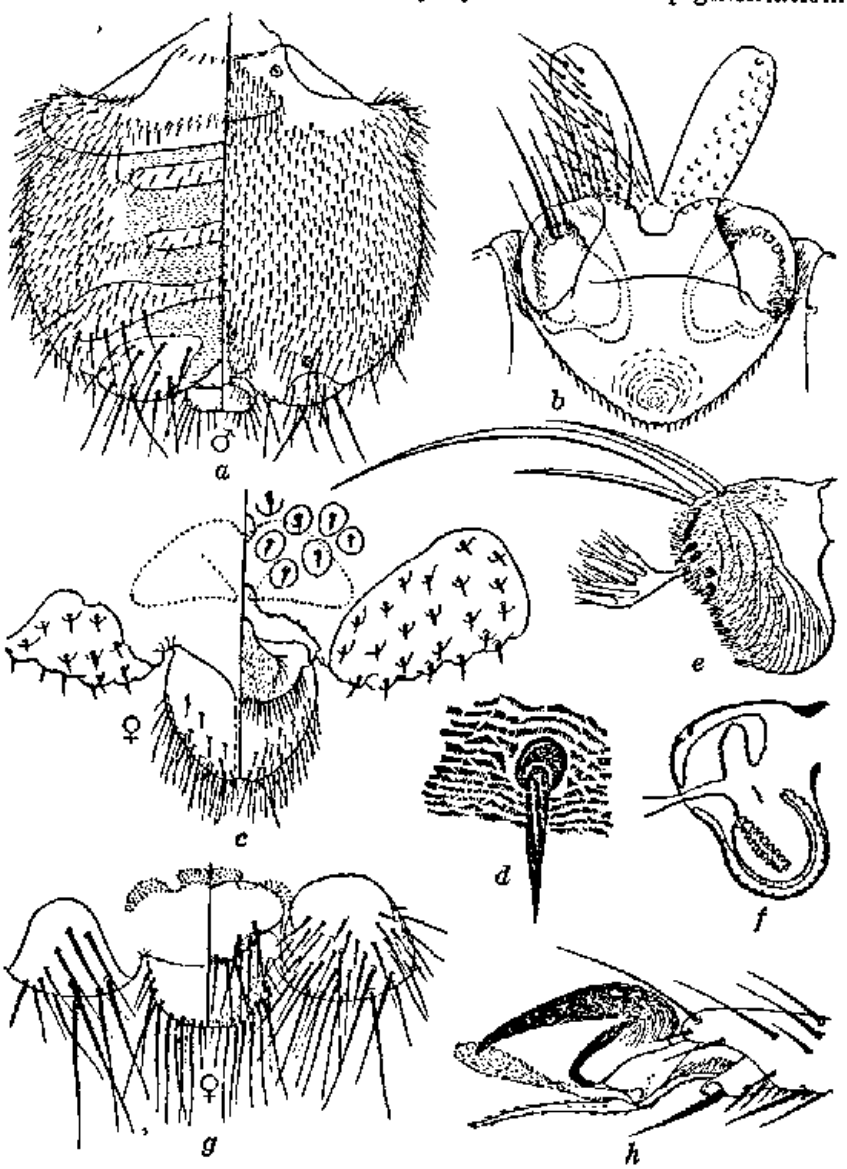

FIG. 4. *Hippobosca equina* Linnæus; *a*, abdomen of male; *b*, anterior portion of head; *d*, acta and markings of derm of abdomen; *e*, lateral aspect of antenna as dissected from its pit; *f*, optical section of antenna; *g*, apex of abdomen of female; *h*, claw. *Hippobosca maculata* Leach; *c*, apex of abdomen of female.

The venation is indicated in the figure in accord with the commonly accepted Comstock-Needham system. It should be noted that the vein R_{2+3} is almost parallel to the vein R_1. The wing membrane is pigmented throughout except for a distinct hyaline furrow along the medial stem and for the cells sc and R_1. There are no setulæ, and the costal border is beset with short stout setæ, with no long hairs.

The abdomen shows the usual large basal tergite. Following it are three small plates and close to the apex two pairs of large, swollen plates bearing long setæ (fig. 4, *g*). The apical lobe of the abdomen is broadly rounded and does not possess a median pale stripe. The ventral side of the abdomen bears only a small basal plate. The setæ of the abdomen are variable in size and each arises from a small, sclerotic base. The derm is marked with minute, sclerotic and pigmented, squamose-reticulate areas (fig. 4, *d*), especially prominent in the median dorsal region of specimens that are not fully expanded, but there is little or no development of such an area of transverse striations as appears in the Olfersiinæ.

The male is very similar to the female, except that the dorsal plates of the abdomen (fig. 4, *a*) are conspicuously larger and the apical pair of marginal plates is lacking. The claspers are very small.

HIPPOBOSCA MACULATA Leach. Figs. 2, *b*; 4, *c*; 5.

STEKHOVEN, Die Bloedzuigende Arthropoda van Nederl Oost Indie 1–2 (1923) 1–82, figs.

Specimens examined.—Numerous individuals from Java, received through the kindness of Dr. J. H. Schuurmans Stekhoven.

Notes.—This species is characteristically a parasite of domestic cattle and has been recorded from Europe, Africa, southern Asia, and the East Indies. Its occurrence in the Philippine Islands is confidently to be expected.

The species is larger and darker in color than *H. equina*, but aside from these differences it is also very distinct structurally. It is one of a group of structurally very similar species that includes at least *H. camelina* Leach (= *H. dromedarina* Speiser) and *H. rufipes* von Olfers. The wing (fig. 2, *b*) is marked especially by the very short R_{2+3}, which appears almost as a crossvein and by the absence of pigmentation from the entire region occupied by the veins. The head (fig. 5) is more nearly circular than in *H. equina*, and the thorax much less hairy.

The scutellum is broad and almost truncate. The abdomen, except for the median dorsal region, is thickly beset with small setæ, each arising from a sclerotic ring. On the ventral side the setæ decrease in length toward the apex and the sclerotic bases become enlarged into rather large tubercles. There are no median tergal plates. The two pairs of subapical lateral plates are very large and prominent (fig. 4, c), the first pair being beset with long setæ and the second only by very short setæ. The apical lobe is of a characteristic form and is divided by a pale median furrow into two parts. The male is very similar to the female, lacking the median tergal plates of the abdomen and having the same subapical, lateral plates and apical lobe. However, the apex of the abdomen is retracted strongly to the ventral side of the body; the first pair of subapical plates is very large and the second pair appears only on the ventral side. The ventral side lacks the area of large tubercles that is seen in the female.

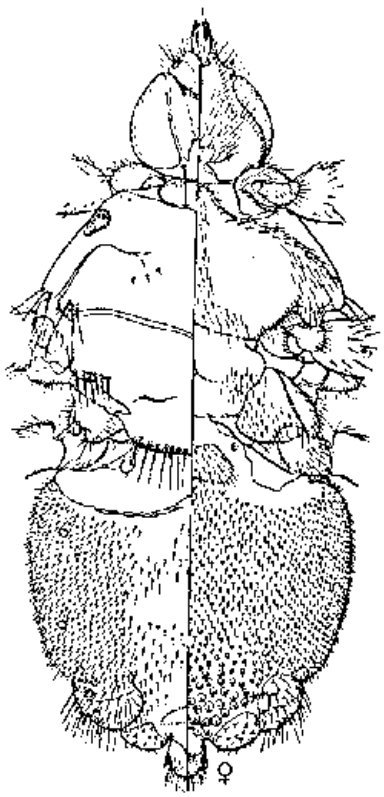

FIG. 5. *Hippobosca maculata* Leach: female, wings and legs removed. From a specimen from domestic cattle in Java.

Genus MELOPHAGUS Linnæus

Hippoboscidæ in which the forewings are represented only by a minute process and the halteres are entirely lacking, the thorax greatly reduced. Claws simple. Antennæ short, retracted into the antennal pits. Eyes reduced, ocelli lacking.

Type of the genus, *Melophagus ovinus* Linnæus.

Notes.—This genus represents the extreme of specialization by reduction of parts to be found in the Hippoboscidæ. I would regard it, however, as belonging to the subfamily Hippoboscinæ.

MELOPHAGUS OVINUS Linnæus.

FERRIS and COLE, Parasitology 14 (1922) 192, figs. 8 and 9.

Notes.—This species is a familiar parasite of sheep and must surely be present in the Philippine Islands. It need not be discussed further here.

Genus LIPOPTENA Nitzsch

FERRIS and COLE, Parasitology 14 (1922) 180.

Hippoboscidæ with the wings well developed and functional throughout an early period of adult life, but eventually caducous and represented in older specimens only by their bases; venation tending to be weak and simplified by the suppression of certain veins, the disappearance of the medial stem leaving a large median cell. Antennæ short, almost completely concealed within their pits. Eyes well developed, although somewhat reduced. Ocelli present. Claws simple. Abdomen without a distinct dorsal, median area of transverse striations.

Type of the genus, *Pediculus cervi* Linnæus.

Notes.—This genus is composed of about a dozen described species, all of which occur on ungulates of the families Cervidæ, Tragulidæ, and Bovidæ. As represented in collections, they are generally wingless with but the wing bases remaining. However, specimens are occasionally taken in flight. Such volant individuals are usually somewhat difficult to associate with their dealated forms; because, as they have not fed, the abdomen is always greatly contracted.

The genus has been discussed at some length by Ferris and Cole (loc. cit.), and the wing of one species has there been figured. The single specimen at hand from the Philippine Islands is winged and throws some additional light upon the character of the venation in these forms. In two species, *L. cervi* (Linnæus) and *L. subulata* Coquillet, the venation is so greatly reduced as somewhat to obscure the pattern, but in the Philippine specimen the venation is more strongly developed (fig. 7, *a*). Here veins R, and R_{2+3} are present, although faint, the principal vein being R_{4+5}. In all the species the medial stem is suppressed, but its position is indicated still by a furrow. The suppression of this stem causes the formation of a single large cell, the apex of which is closed by the radial-medial crossvein and the branches of media, the whole appearing as a single oblique crossvein. It is evident that the venation is not of so isolated a type as has been supposed.

I would regard the genus as a member of the Hippoboscinæ. Its nearest relative is *Echestypus*, from African ungulates, which differs chiefly in the absence of ocelli and in the much reduced palpi. In addition to the following species it is possible that one is to be found on goats in the Philippine Islands.

LIPOPTENA sp. Figs. 6 and 7, a.

Specimen examined.—A single volant female from Mount Maquiling, Laguna Province, Luzon (*C. F. Baker*). The hosts are in all probability deer of the genus *Rusa*.

Notes.—The extreme contraction of the abdomen in volant individuals makes it impossible to determine definitely the characteristics of this part of the body, while in most species the head and the thorax offer but little in the way of definite specific characters. Thus, while there are slight differences between the specimen at hand and the types of *L. traguli* Ferris and Cole (which is probably a synonym of *L. gracilis* Speiser) which lead to a belief that the two species are distinct, I hesitate to describe this species as new on the basis of this single specimen. However, I am here figuring the species in order to call attention to the presence of the genus in the Philippine Islands.

The specimen is somewhat larger than the types of *L. traguli*, its length being 4 millimeters and the wing 4 millimeters; the length of an expanded specimen of *L. traguli* is the same. There is an indication of the presence of at least one small abdominal tergal plate which is not present in *L. traguli*.

Genus CRATAERINA von Olfers

AUSTEN, Parasitology 18 (1926) 350.

Hippoboscidæ belonging to the Ornithomyiinæ, with the wings present but reduced in size, at the most but little exceeding the apex of the abdomen, although retaining the normal number of well-developed veins, and with the apical portion of the wing strongly narrowed.

Type of the genus, *Ornithomyia pallida* Latreille (= *Crataerina lonchoptera* von Olfers).

Notes.—The genera *Crataerina*, *Myiophthiria*, and *Brachypteromyia* have been recognized for the reception of species having reduced wings and all occurring on birds of the family Micropodidæ. I have elsewhere shown that *Brachypteromyia* cannot be maintained as distinct from *Myiophthiria*. I am further strongly inclined to the view that the latter genus should be merged with *Crataerina*. There can be no doubt that these

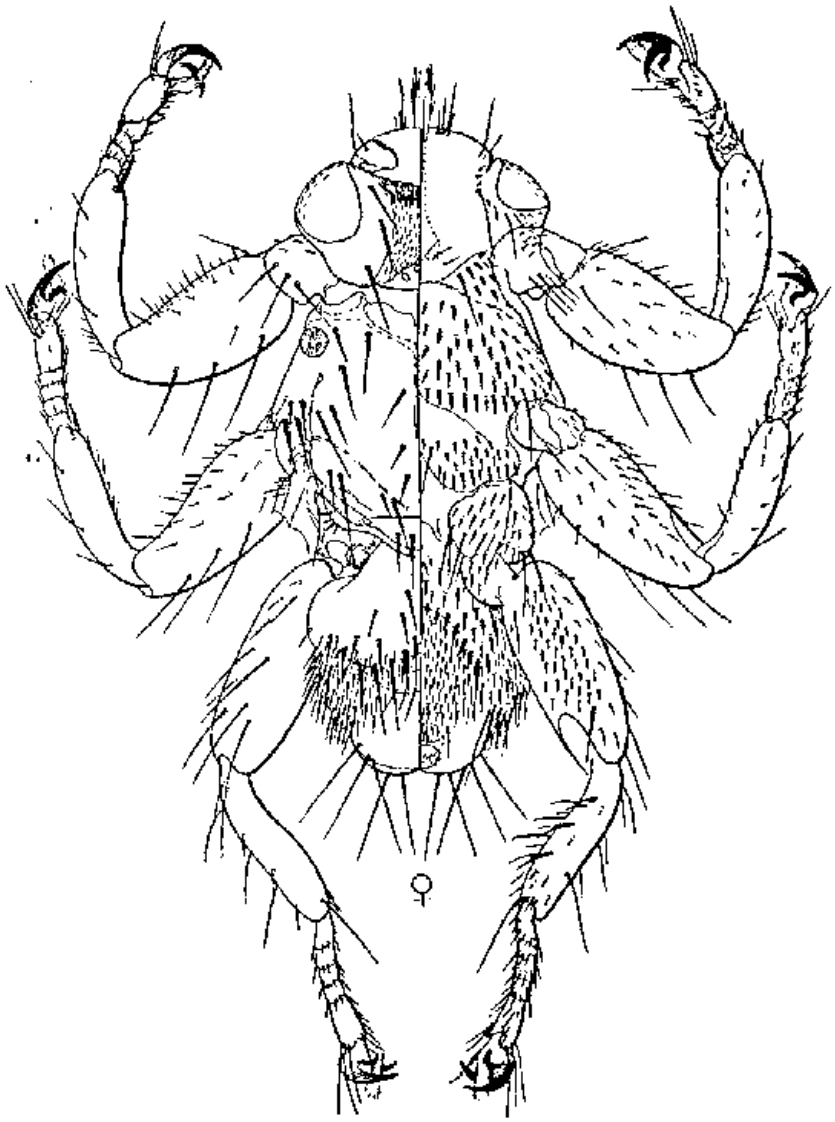

FIG. 6. *Lipoptena* sp.; female.

two so-called genera are members of a single stock of very closely related forms.

The only basis for a generic separation is to be found in the character of the wings. In *Myiophthiria* these are greatly re-

duced, not attaining even to the middle of the abdomen, and with the venation reduced to two or three longitudinal veins in addition to the strong costa. In *Crataerina*, on the other hand, the reduction of the wings is chiefly by way of a narrowing of the wing membrane; the veins, while somewhat reduced and much crowded, are still of about the usual number; the whole structure exceeds the tip of the abdomen. In one species, however, *C. obtusipennis* Austen, they are described as resembling the wings of *Myiophthiria* in form, although retaining the typical venation.

It seems evident that while there may be a technical basis for the recognition of two genera, all these species constitute a single series with *Myiophthiria* (= *Brachypteromyia*) *fimbriata* (Waterhouse), in which the wings are very small and the venation is reduced almost to a single vein, at one end, and *Crataerina longipennis* Austen, in which the wings are long and slender and the venation complete, at the other.

The question is merely one of what concept of the genus shall be adopted. I am inclined to accept the concept that calls for broader groups, but it would be well to examine certain apparently annectant forms, such as *Myiophthiria lygaeoides* and *Crataerina obtusipennis*, before definitely advocating the fusion of these two genera.

I have discussed the genus *Myiophthiria* in an earlier paper of this series. The single species here referred to *Crataerina* is not from the Philippine Islands, but closely related forms, or possibly even this species, must occur in these islands.

CRATAERINA ACUTIPENNIS Austen. Fig. 7, b.

AUSTEN, Parasitology (1926) 355, text fig. 1a.

Previous records.—From Madeira, Canary Islands, and South Africa, on various species of swifts.

Specimen examined.—A single female from "*Cypselus affinis*," Ceylon, received through the kindness of Mr. E. E. Green.

Notes.—A figure of this species would in most respects be merely a duplicate of the figure of *Myiophthiria reduvioides*, which has been presented in an earlier paper of this series. It is larger than the latter, pale in color, the legs are noticeably stouter, and it is perhaps somewhat more hairy, but otherwise there is little difference except for the wings, which slightly exceed the apex of the abdomen. The wing is shown in fig. 7, *b*.

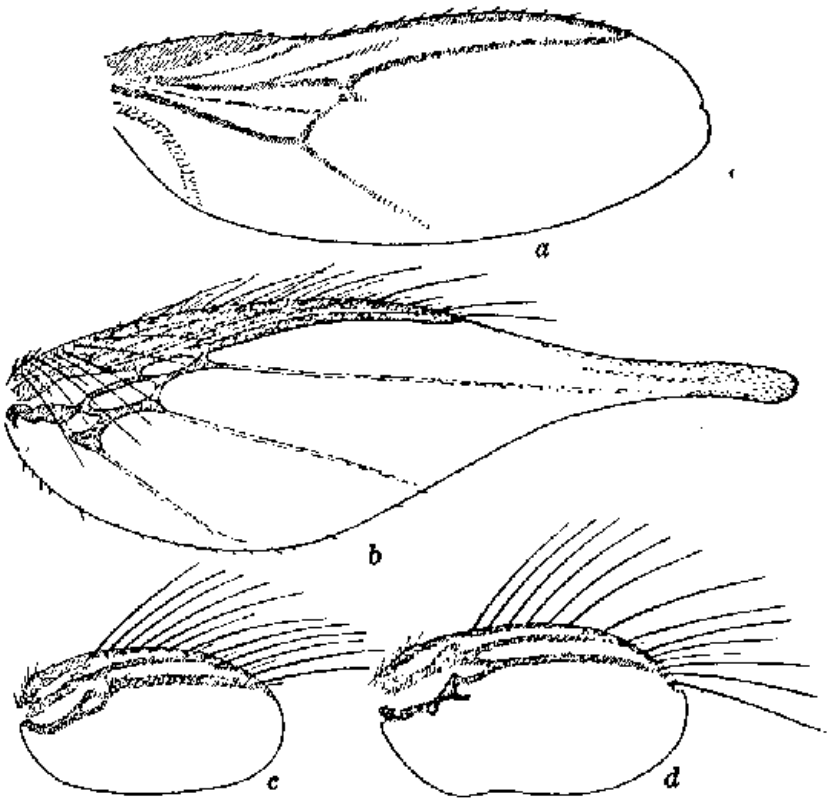

FIG. 7. *Lipoptena* sp.; *a*, wing. *Crataerina acutipennis* Austen; *b*, wing, from a specimen from Ceylon. *Myiophthiria reduvioides* Rondani; *c* and *d*, wings, from specimens from the Philippine Islands, showing variations.

Genus MYIOPHTHIRIA Rondani

MYIOPHTHIRIA REDUVIOIDES Rondani. Figs. 7, c and d.

FERRIS, Philip. Journ. Sci. 28 (1925) 377, fig. 5; 34 (1927) 218, fig. 9.

Additional records.—In addition to previous records, are the following: One male, host unknown, Badajos, Tablas Island (*F. Rivera*); and one female from *Geopelia striata* (Linnæus), Novaliches, Rizal Province, Luzon (*R. C. McGregor*).

Notes.—I may here call attention to the variation in the wings in specimens that I consider to represent this species. In fig. 7, *c*, is the wing of the specimen from Badajos and in fig. 7, *d*, that from Novaliches. These are drawn to the same scale and it may be noted that there is an evident variation in size as well as in the venation. Other specimens differ more or less from those here figured.

Genus ORNITHOICA Rondani

FERRIS, Canadian Entomologist 61 (1929) 280.

Certain errors for which I have been responsible in my previous treatment of this genus are pointed out in the reference cited above. Briefly, it may be said that owing to a failure properly to associate males and females the two sexes of a single species are recorded under different names. The corrections are noted below.

ORNITHOICA PUSILLA (Schiner).

Ornithoica promiscua Ferris and Cole, FERRIS, Philip. Journ. Sci. 28 (1925) 331–332.

Ornithoica pusilla (Schiner), FERRIS, Philip. Journ. Sci. 34 (1927) 207.

Ornithoica pusilla (Schiner), FERRIS, Canadian Entomologist 61 (1929) 28.

Additional records.—Females from *Pseudoptynx philippinensis* Kaup, Cavite Province, Luzon, July 30, 1928 (*R. C. McGregor*); and one male from *Loriculus bournsi* McGregor, Tablas Island, August 30, 1928 (*F. Rivera*).

Notes.—I have previously failed on the one hand properly to appreciate the specific differences between the American *Ornithoica confluenta* (Say) (= *O. promiscua* Ferris and Cole) and *O. pusilla* (Schiner) and on the other to associate male and female of the same species. I have consequently recorded females from the Philippine Islands as *O. promiscua* and their males as *O. pusilla*. That these males and females belong together seems clear, although even yet I have not seen specimens of the two sexes from the same host. The species may be accepted as *O. pusilla*.

Genus ORNITHEZA Speiser

ORNITHEZA METALLICA (Schiner).

FERRIS, Philip. Journ. Sci. 27 (1925) 419, figs. 4, 5; 34 (1927) 213, fig. 6.

Additional records.—A male from *Xantholæma roseum* (Dumont), and two females from *Loriculus bournsi* McGregor, Badajos, Tablas Island, August 29, 1928 (*F. Rivera*); a female from *Eurystomus orientalis* (Linnæus), Davao, Mindanao, March 27, 1927 (*F. Rivera*); a male from *Hemiprocne comata* (Temminck), Mayo, Mindanao, April 21, 1927 (*F. Rivera*).

Notes.—The specimen from *Eurystomus* differs from others in lacking the small dorsal plates of the abdomen, but their position is indicated by hairless areas.

Genus ORNITHOMYIA Latreille

ORNITHOMYIA AVICULARIA (Linnæus).

FERRIS, Philip. Journ. Sci. 34 (1927) 211, figs. 4 and 5.

Additional record.—A single female from *Pyrotrogon ardens* (Temminck), Mount Mayo, Davao, Mindanao, April 25, 1927 (*F. Rivera*).

Genus LYNCHIA Weyenbergh

LYNCHIA SARTA (Ferris).

Ornithoponus sartus FERRIS, Philip. Journ. Sci. 28 (1925) 333, figs. 3 and 4.

Additional record.—A single female from *Microhierax meridionalis* Grant, Mount Galintan, Davao, Mindanao, May 14, 1927 (*F. Rivera*).

LYNCHIA SETOSA Ferris.

FERRIS, Philip. Journ. Sci. 34 (1927) 227, figs. 14 and 15.

Additional records.—A male from *Circus* sp., Manila, February 14, 1927 (*R. C. McGregor*); and a male from *Ixobrychus astrologus* Wetmore, Obando, Bulacan Province, Luzon, December 16, 1927 (*R. C. McGregor*).

ILLUSTRATIONS

TEXT FIGURES

VI. NACHTRAG ZUR KENNTNIS DER PHILIPPINISCHEN RUTELIDEN (COLEOPTERA, LAMELLICORNIA)

Von F. Ohaus

Mainz, Germany

FIER FIGUREN

Kürzlich erhielt ich von den Herren Dr. O. Staudinger und A. Bang-Haas, Dresden-Blasewitz, eine grössere Sendung Rutheliden zur Determination zugeschickt, darunter auch die Doubletten derjenigen Arten, welche Herr Georg Böttcher während seiner Reisen auf den Philippinen gesammelt hatte. Unter diesen entdeckte ich mehrere neue Arten—leider nur in je 1 Exemplar—deren Beschreibung ich hiermit bekannt gebe.

PARASTASIA NIGROSCUTELLATA Ohaus.

Von dieser seltenen Art, von der ich bisher nur zwei Exemplare in die Hände bekam, sammelte Herr O. Schütze in San Antonio bei Laguna, Luzon, einen ♂, bei welchem die ganze Unterseite, Afterdecke und Beine schön rotgelb sind; oben ist der Thorax rotgelb mit zwei kleinen schwarzen Flecken, der ganze Kopf, das Schildchen und die Deckflügel einfarbig schwarz. Forceps wie bei der Nominatform.

POPILLIA FURCULA sp. nov.

Der *macronyx* Ohaus zunächst verwandt, oval, flach gewölbt, hinter den Schultern verbreitert und nach hinten verschmälert. Oben und unten nebst den Beinen gleichmässig glänzend schwarz; oben die Umrandung des Thorax, der Hinterrand des Propygidiums und vier scharf begrenzte Fleckchen auf dem Pygidium, unten eine Querreihe auf den Abdominalsterniten und die Seiten sowie die Epimeren der Hinterbrust mit weissen Schuppenhaaren. K o p f schild trapezförmig, wie die flach eingedrückte Stirn dicht runzelig,

Fig. 1. *Popillia furcula* sp. nov., Forceps.

Scheitel mehr einzeln punktirt. Halsschild vorn und an den Seiten dicht und grob zusammenfliessend punktirt, mit Bogenstricken, die um die hintere Parthie der Scheibe herumlaufen, diese selber sowie die Parthie vor dem Schildchen fein einzeln punktirt; ebenso ist das Schildchen punktirt. Auf den Deckflügeln ist die Parthie neben den stark vorspringenden Schultern und die Scheibe flacher eingedrückt als bei der *macronyx*, die primären Punktreihen sind fein gefurcht, Rippen und Interstitien kaum gewölbt. Propygidium dicht und fein einzeln punktirt. Pygidium mit grossen Punkten, die nur auf der Spitze einzeln stehen, sonst in quer verlaufende Bogenlinien zusammenfliessen; in den Vorderecken je ein kleines Grübchen. Auf den Bauchringen ist die Querreihe von Borstenpunkten in der Mitte unterbrochen, die weissen Schuppenhaare an den Seiten dichter stehend. Mesosternalfortsatz gross und kräftig, seitlich zusammengedrückt, etwas gesenkt und gerundet. Beine und Füsse kräftig. Am Forceps, Fig. 1, sind die breiten flachen Parameren vorn gleichmässig zugespitzt; die Ventralplatte des Mittelstücks ist wie ein Unterschnabel nach oben gekrümmt, die Spitze breit gegabelt.

Länge 9.5 bis 10 mm., Breite 5.5 bis 6 mm. ♂ ♀. Süd-Luzon (*G. Böttcher*).

ANOMALA MURICATA sp. nov.

Aus der Gruppe der *A. ovatula* Ohaus. Kurz eiförmig, gut gewölbt, dunkelbraun mit erzgrünen und kupfrigen Lichtern, lebhaft glänzend, oben kahl, unten spärlich und kurz gelb behaart. Kopfschild trapezförmig mit kräftig aufgebogenem Rand, Stirnnaht in der Mitte eingedrückt, die Stirn abgeflacht, wie Kopfschild dicht mit kräftigen zusammenfliessenden Punkten bedeckt, kupfrig, während der erzgrüne Scheitel mit kräftigen einzelnen Punkten dicht besetzt ist. Thorax mit Seitengrübchen und feiner Randfürche ringsum, wie Scheitel und Schildchen einzeln dicht grob punktirt. Auf den Deckflügeln sind die primaren Punktreihen tief gefurcht, Rippen und Interstitien gleich hoch gewölbt, im subsuturalen Interstitium zwei secundäre Rippe, die durch eine vorn unregelmässige Punkti-

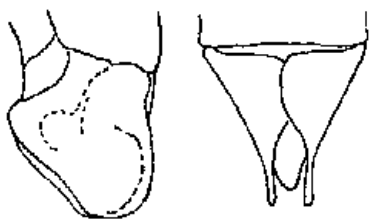

Fig. 2. *Anomala muricata* sp. nov., Forceps.

rung breit getrennt sind, im II. und III. Interstitium zwei, in den seitlichen Interstitien je eine secundäre Rippe. Pygidium ziemlich flach und seitlich nebender Spitze etwas eingedrückt, mit grossen in die Quere gezogenen und zusammenfliessenden Punkten; Spitze und Seiten mit einzelnen Borsten; ebenso ist die Unterseite sculptirt und behaart. Fühler kräftig, gelb, die keule so lang als die Geissel. Forceps Fig. 2.

Länge 10.5 mm., Breite 6.5 mm. ♂. Palawan (*Staudinger*).

ANOMALA NOCTURNA sp. nov.

Aus der Gruppe der *A. leotaudi* Blanchard. Länglich eiförmig, gewölbt. Oben und unten dunkel rotbraun, glänzend, auf Kopf, Halsschild und Schildchen mit grünem Erzschimmer, die Fühler braungelb. Kopfschild trapezförmig, der schwarze Rand hoch aufgebogen, die Fläche wei die abgeflachte Stirn mit groben, zusammenfliessenden Punkten dicht bedeckt, kupferig, während der Scheitel mit einzelnen kräftigen Punkten überstreut erzgrün ist. Thorax ringsum fein gerandet, ohne Seiten grübchen, wie das Schildchen überall dicht und stellenweise zusammenfliessend punktirt. Auf den Deckflügeln sind die primären Punktreihen fein gefurcht, die Rippen nicht höher gewölbt als die Interstitien, im subsuturalen Interstitium eine dichte unregelmässige Punktirung, im II und III. Interstitium je eine einfache Punktreihe, die Punkte vielfach quer eingedrückt, das Gewebe zwischen ihnen zu feinen Querrunzeln erhoben und hierdurch sowie eine Menge feiner Pünktchen die Sculptur etwas undeutlich; der Seitenrand mit langen, abstehenden braunen Borsten. Pygidium dicht querrunzelig, am Rand und auf der Spitze mit langen Borsten. Unterseite dicht runzelig punktirt, die Brust dicht, die Bauchseiten und Beine spärlicher braun behaart. An den Vorderschienen ist der Seitenzahn hinter dem Spitzenzahn kräftig; Mittel- und Hinterschienen mit zwei schiefen Stachelkanten. An den Vorderfüssen trägt die grössere innere Klaue an der dorsalen Kante vor der Spitze eine Borste, die Spitze ist einfach; an den Mittelfüssen ist die grössere äussere Klaue an der Spitze ganz schwach eingeschnitten; an den Hinterfüssen sind beide Klauen einfach,

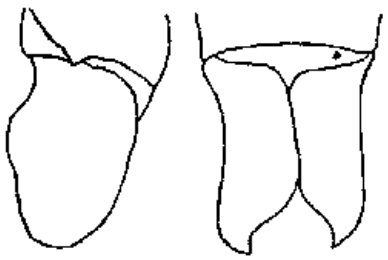

FIG. 8. *Anomala nocturna* sp. nov., Forceps.

nahezu gleich lang. Die Fühlerkeule ist so lang als die Geissel. Am Forceps, Fig. 3, sind die einfachen kleinen Parameren symmetrisch.

Länge 11.5 mm., Breite 6.5 mm. ♂. LUZON, Baguio (*G. Böttcher*).

EUCHLORA NERISSA sp. nov.

Der *E. ceramopyga* Ohaus zunächst verwandt. Länglich eiförmig, mässig gewölbt. Oberseite gleichmässig blattgrün, die Seiten des Thorax schmal gelb; Pygidium gleichmässig erzgrün; Unterseite, Schenkel und Fühler hell rötlichgelb, die Schienen und Füsse erzgrün. Pygidium ganz kahl; Abdominalsternite mit einer Querreihe von Borstenpunkten; die Brust ganz

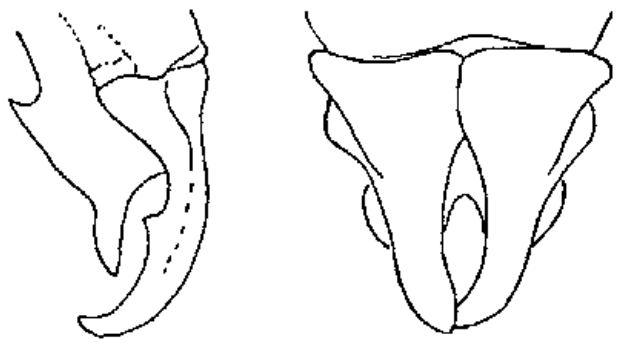

FIG. 4. *Euchlora nerissa* sp. nov., Forceps.

spärlich und kurz behaart. Kopfschild und Stirn fein zusammenfliessend punktirt, der Scheitel mit einzelnen feinen Pünktchen. Thorax ziemlich dicht bedeckt mit Pünktchen, die in der Mitte sehr fein, an den Seiten etwas stärker sind. Die Deckflügel sind sehr glänzend, wie mit einem feinen Firnis bedeckt und tragen überall sehr feine, nur unter der Lupe sichtbare Pünktchen; neben der Schülter–Apicalbuckel–linie eine Reihe kürzer Querfalten. Pygidium dicht bedeckt mit Hufeisenpunkten, die der Quere nach zusammenstossen, Querrunzeln und hier und da kleine Höckerchen bilden. Abdominalsternite an den Seiten fein runzelig punktirt. Am Forceps, Fig. 4, tragen die Parameren in der Mitte der Unterseite einen Zahn.

Länge 18.5 mm., Breite 10.5 mm. ♂. MINDANAO, Zamboanga (*G. Böttcher*).

ALKALOID FROM ANONA RETICULATA LINNÆUS

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Many members of the family Anonaceæ have been found to contain alkaloids. De Rochebrune [1] isolated from the fruits of *Xylopia etiopica* A. Rich. an alkaloid crystallizing in long fine prisms which he called anonaceine. De Rochebrune [2] mentions the occurrence of anonaceine in several plants belonging to the Anonaceæ. J. Marañon [3] isolated artabotrine ($C_{36}H_{38}O_6N$), melting point, 187° C., from the bark of *Artabotrys suaveolens* Blume.

In the genus *Anona*, there are three species found in the Philippines; two of these have been reported to contain alkaloids. Callan and Tutin [4] in the process of a chemical examination of the leaves, reported the presence of an amorphous alkaloid in *Anona muricata* Linnæus. N. Trimurti [5] found in the leaves of *Anona squamosa* Linnæus a white powdery base which occurred in the amount of 0.4 per cent calculated as chloroplatinate.

According to the literature there is no work done on the isolation of an alkaloid from *Anona reticulata* Linnæus, the third species of *Anona*, a tree that is extensively cultivated in the Philippines. Therefore, it was thought that it would be interesting to find out if, as expected, this species also contained an alkaloid. The plant is commonly known as "anonas." According to Pardo de Tavera,[6] the green fruit is used to check diarrhœa and dysentery on account of the large quantity of tannin it contains. The juice of the trunk is irritant. The leaves and the fruit of *Anona reticulata* Linnæus are official in the first

[1] Pharm. Rundschau Nr. 34 (1901), through Pharm. Ztg. 46 (1901) 693–694.

[2] Toxicologie Africaine 1 (1897) 385.

[3] Philip. Journ. Sci. 38 (1929) 259–265.

[4] Phar. Journ. IV 33 (1911) 743.

[5] Jour. Ind. Inst. Sci. 7 (1924) 232–234, through Chem. Abs. 1 (1925) 656.

[6] Plantas Medicinales de Filipinas, Madrid (1892) 22.

to the fourth edition of the Mexican Pharmacopœia.[1] In the present investigation, an alkaloid melting at 122 to 123° C. was isolated from the bark of the trunk of *Anona reticulata* Linnæus grown in the neighborhood of Manila. From the results of the elementary analysis, molecular-weight determination, and analysis of the hydrochloride the alkaloid corresponds to the formula $C_{17}H_{15}NO_3$. It does not contain a methoxyl group. Of the three oxygen, two are in the form of a dioxymethylene group. In order to distinguish the alkaloid from those obtained by previous investigators from other plants of the family Anonaceæ the writer proposes to name it "anonaine."

The bark examined was found to contain 0.03 per cent anonaine. Because of the small yield and the small amount of the alkaloid that was obtained, the writer discontinued the work temporarily.

EXPERIMENTAL

A quantity of the powdered, air-dried bark weighing 1.8 kilograms was refluxed three times with the same volume of 95 per cent alcohol on the water bath for about three hours. The alcohol was recovered from the combined alcoholic extracts at first by distillation, afterwards by evaporation on a water bath, until the odor of alcohol was no longer perceptible. The sirupy brownish residue was acidified distinctly with acetic acid and treated with water. This treatment caused a separation of a resinous matter.

The aqueous acid solution was shaken out with ether and rendered alkaline with ammonia. The ethereal solution was shaken with 5 per cent sodium hydroxide solution repeatedly until no more phenolic bases went into the alkali. On treating the ethereal solution with dilute hydrochloric acid, the hydrochloride crystallized out. This was filtered, recrystallized, again dissolved in water, shaken out with ether, and alkalinified with ammonia. On evaporation of the ether the alkaloid crystallized in long needles melting at 122 to 123° C. The yield was about 0.25 gram.

The sodium hydroxide solution was acidified, shaken out with ether, and made alkaline with ammonia. On evaporation of the ether there remained about 0.1 gram residue. On account of its small quantity it was not studied further.

[1] Bruntz, L., and M. Jaloux, Plantes Medicinales et Plantes a Drogues Medicamenteuses. Paris (1918) 68.

To recover the alkaloid contained in the resinous matter, the material was dissolved in dilute sodium hydroxide and the alkaloid extracted shaken out with ether. When the ether extract was treated with dilute hydrochloric acid the insoluble hydrochloride separated out again. It was treated further as above. The yield from the resin was about 0.33 gram. More than one-half of the alkaloid was thus contained in the resin.

From 1.8 kilograms of bark about 0.7 gram of alkaloid was obtained. The alkaloid is slightly levorotatory.

0.0836 gram of substance dissolved in 10 cubic centimeters of chloroform gave a specific rotation $[\alpha]_D^{32.5^\circ C.}$ of -83.01°.

The base was dried to constant weight over calcium chloride in a vacuum:

Analysis:

Analysis No.—	Substance.	Carbon dioxide.	Water.	Nitrogen.
	mg.	*mg.*	*mg.*	*cc.*
1	5.920	15.681	2.956	
2	5.864	15.592	3.217	
3	5.955	15.904	3.052	
4	6.135			0.276 (34° C; 761 mm.)
5	5.943			0.247 (32° C; 762 mm.)

	Carbon.	Hydrogen.	Nitrogen.
	Per cent.	*Per cent.*	*Per cent.*
Calculated for $C_{17}H_{15}NO_2$	72.3	5.7	5.0
Found	72.2	5.6	4.9
	72.5	6.1	4.8
	72.8	5.7	

Molecular weight determination according to Rast gave the following results:

Experiment No.—	Substance.	Camphor.	Depression.
	mg.	*mg.*	*°C.*
1	1.270	9.877	18
2	2.592	18.436	20.5

	Molecular weight.
Calculated for $C_{17}H_{15}NO_2$	282
Found	285.7
	274.4

The hydrochloride crystallizes from water in the form of fine silky needles. It did not lose weight on drying over phosphorus pentoxide (80°C) in a vacuum.

Analysis:

63.803 mg substance gave 28.460 mg AgCl

	Chlorine. Per cent.
Calculated for $C_{11}H_{15}NO_2HCl$	11.0
Found	11.1

The chloroplatinate was obtained as a yellow amorphous precipitate by treating a solution of the hydrochloride, acidified with hydrochloric acid, with a slight excess of chloroplatinic acid. It does not crystallize either from alcohol or dilute acids. It was filtered and washed with water acidulated with hydrochloric acid.

The chloroplatinate did not lose weight on drying over phosphorus pentoxide (80°C) in a vacuum:

Analysis:

Analysis No.—	Substance.	Platinum.
	mg.	mg.
1	57.778	11.805
2	45.225	9.305

	Platinum. Per cent.
Calculated for $(C_{11}H_{15}NO_2HCl)_2PtCl_4$	20.04
Found	20.43
	20.57

	Molecular weight of free base.
Calculated for $C_{11}H_{15}NO_2$	282.0
Found	272.4
	269.3

Methoxyl determination according to Zeisel-Pregl gave negative results. The qualitative detection of a dioxymethylene group according to Gaebel showed distinctly the presence of a methylene dioxide group.

ACKNOWLEDGMENT

The author wishes to express his thanks to Dr. Leon M. Guerrero for suggesting this investigation on the Anonaceæ.

CARBOHYDRATE AND SEROLOGICAL DETERMINATIONS OF THE BIPOLAR GAS-FORMING AND NON-GAS-FORMING ORGANISMS ISOLATED FROM LYMPH GLANDS OF SLAUGHTERED CATTLE

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In 1927 three strains of gas-forming bipolar organisms were reported.[1] They were isolated from slaughtered cattle. The investigation was extended with a view to find other types of these organisms, taking advantage of the ease of isolating them in pure culture from such organs. The first sample of glands investigated was from a cow which came from Palawan Province, and the second sample was from a cow slaughtered at Sisiman Slaughter House. The strain isolated from the first sample was labeled G–4 and that from the second sample G–5. These two strains were studied and compared with the three strains of bipolar organism that were previously reported.

The procedure of isolation was the same as given in the previous report; that is, the gland was dissected, sterilized superficially, and cut; the internal portion was scraped, triturated in a sterile mortar, and diluted with salt solution. One cubic centimeter of this solution was injected subcutaneously. Upon the death of the animal cultures were made from the heart, the liver, and the spleen.

The strain G–4 (Table 1) was a gas-forming bipolar organism and presented cultural characteristics similar to those of the three strains previously reported (Table 1, G–1, G–2, and G–3). The strain G–5 (Table 1) showed the following biochemical characteristics. It was a non-gas-forming bipolar organism,

[1] Phillip. Journ. Sci. 33 (1927) 331.

TABLE 1.—*Showing the carbohydrate reactions.*

[+, acid only; ++, acid and gas; F, faintly acid; 0, negative.]

Carbohydrates, 1 per cent.	Peptone-salt solution with Andrade indicator in Durham's fermentation test tubes.														
	April 29, 1927.					May 2, 1927.					May 5, 1927.				
	G-1	G-2	G-3	G-4	G-5	G-1	G-2	G-3	G-4	G-5	G-1	G 2	G-3	G-4	G-5
Glucose	+	+	+	+	+	++	+	++	++	+	++	+	++	++	+
Galactose	+	+	+	+	+	++	+	++	++	+	++	+	++	++	+
Levulose	+	+	+	+	+	++	+	+	+	+	++	+	+	+	+
Arabinose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Xylose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhamnose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Maltose	+	+	+	+	+	++	+	+	+	+	++	+	+	+	+
Lactose	0	F	F	0	F	0	F	F	0	F	0	0	0	0	0
Sucrose	0	0	0	0	+	0	0	0	0	+	0	0	0	0	+
Amygdalin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salicin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Raffinose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dextrin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sorbitol	+	+	+	+	+	++	++	++	++	+	++	++	++	++	+
Dulcitol	+	+	+	+	0	++	++	++	++	0	++	++	++	++	0
Mannitol	0	0	0	0	+	0	0	0	0	+	0	0	0	0	+
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Adonitol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Inositol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nutrose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspargin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Inulin	+	+	+	+	+	++	++	++	++	+	++	++	++	++	+
Erythritol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Gram-negative, and nonmotile. It grew on blood agar heated to 70° C. and showed a translucent, slightly elevated, grayish colony; the pheriphery was regular. It produced a uniform turbidity in ordinary bouillon which occasionally showed a very thin pellicle after four or five days incubation. Sediment was formed at the bottom a little later. On ordinary acid agar, if it grew at all, the growth was slow and scanty, similar to that of streptococcus. There was reduction in nitrate broth.[2] The nitroso-indol reaction was positive in forty hours. No gas developed in the Smith fermentation tube containing infusion broth to which 1 per cent glucose was added.

Rabbits and guinea pigs died within two or three days after inoculation with living cultures. The two strains (G–4 and G–5) did not grow on eosin-methylene blue-lactose agar.

In order to establish the identity of the two newly isolated strains (G–4 and G–5) with those that were already reported (G–1, G–2, and G–3) the following studies were made: First, the study of their behavior towards various sugars; second, the study of the gas production in culture media; and third, serological study.

BIOCHEMICAL REACTIONS

The five strains were inoculated in Dunham's solution containing 1 per cent of various carbohydrates with 1 per cent Andrade's indicator adjusted to such a reaction that when heated it turned pink and became colorless when cool. Normal solution of sodium hydroxide was used to adjust this reaction.[1]

As seen in Table I, the strains G–1, G–2, G–3, and G–4 fermented glucose, galactose, levulose, maltose, sorbitol, dulcitol, inulin, and mannose. There was gas formation in most of the fermented sugars. The strain G–5 fermented glucose, galactose, levulose, maltose, sucrose, sorbitol, mannitol, inulin, and mannose. It did not produce gas after incubation for one week.

Sugar-free infusion broth was planted along with Dunham's solution. The results of the sugar reactions in both basic media were the same. However, the formation of gas was evidently more pronounced in the infusion broth than in Dunham's solu-

[1] One per cent peptone was used instead of 0.1 per cent.

[2] In the Philippine Journal of Science 33 (1927) page 338, third line, *for* 0.1 *N. read* 1.0 *N.*

TABLE 2.—*Showing carbohydrate reactions with the subsequent formation of gas in two basic media.*

[+, acid only; ++, acid and gas; 0, negative; B, bubble; n, not used.]

Carbohydrate, 1 per cent.	Media.	Sugar-free infusion broth, peptone salt water, with Andrade indicator in Durham fermentation tube. Inoculated August 20, 1927.																				
		August 21, 1927.					August 24, 1927.					August 27, 1927.					September 1, 1927.				
		G-1	G-2	G-3	G-4	G-5	G-1	G-2	G-3	G-4	G-5	G-1	G-2	G-3	G-4	G-5	G-1	G-2	G-3	G-4	G-5
Glucose Merck	Infusion	+	+	+	+	+	+	++	++	++	+	+	++	++	++	+	+	++	++	++	+
	Peptone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose Pfanstiehl	Infusion	+	+	+	+	+	++	++	++	++	+	++	++	++	++	+	++	++	++	++	+
	Peptone	+	+	+	+	+	++	+	+	++	+	++	+	+	++	+	++	++	++	++	+
Mannite Merck	Infusion	0	0	0	0	+	0	0	0	0	+	0	0	0	0	+	++	+	+	++	+
	Peptone	0	0	0	0	+	0	0	0	0	+	0	0	0	0	+	0	0	0	0	+
Saccharose Merck	Infusion	0	0	0	0	+	0	0	0	0	+	0	0	0	0	+	0	0	0	0	+
	Peptone	0	0	0	0	+	0	0	0	0	+	0	0	0	0	+	0	0	0	0	+
Sorbite Pfanstiehl	Infusion	+	+	+	+	+	B	+	B	B	+	++	++	++	++	+	++	++	++	++	+
	Peptone	+	+	+	+	+	++	+	++	++	+	++	+	++	++	+	++	+	++	++	+
Dulcite Pfanstiehl	Infusion	+	+	+	+	0	++	+	+	++	0	++	++	B	++	0	++	++	+	++	0
	Peptone	+	+	+	+	0	+	+	+	+	0	+	+	+	+	0	+	+	n	n	n
Levulose Pfanstiehl	Infusion	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
	Peptone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose Eimer and Amend	Infusion	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Peptone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannose Merck	Infusion	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
	Peptone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

tion. The number of tubes showing gas was, of course, less in the latter than in the former basic medium (Table 2). It was observed that carbohydrates in fermentation test tubes stored in the ice box for more than one month gave, when inoculated, irregular fermentation reactions. Therefore, sugars with the indicator herein employed should be used within one week.

TABLE 3.—*Showing the result of gas production in Smith fermentation tube. The closed arm measures 1.2 by 10 centimeters. One per cent carbohydrate with no indicator. Autoclaved fifteen minutes at 15 pounds. Result of one week incubation.*

[— X, no gas, the growth reaches the tip of the closed arm: —0, no gas, the growth does not reach the tip of the closed arm: fraction and X, amount of gas by fraction of a centimeter with growth to the tip of the closed arm.]

Strain.	Glucose.		Maltose.		Mannite.		Sorbite.		Galactose.		Dulcite.		Saccharose.	
	Infusion.	Peptone.	Infusion.	Peptone.	Infusion.	Peptone.	Infusion.	Peptone.	Infusion.	Peptone.	Infusion.	Peptone.	Infusion.	Peptone.
G-1	—X	—0	0.6X	—0	—0	—0	—X	—0	—0	—0	0.6X	—0	—0	—0
G-2	—X	—0	—X	—0	—0	—0	—X	—0	—0	—0	0.5X	—0	—X	—0
G-3	—X	—0	—X	—X	—0	—0	0.2X	—0	—X	—0	0.3X	—X	—0	—0
G-4	—X	—0	0.5X	0.2X	—0	—0	0.2X	—0	—X	—0	0.5X	—0	—0	—0
G-5	—X	—0	—X	—0	—X	—0	—X	—0	—X	—0	—X	—0	—X	—X

GAS FORMATION

The evolution of gas was also studied in the Smith fermentation tube without indicator (Table 3). Here again the amount of gas was constantly greater in the tubes that contained sugar-free infusion broth. The maximum length of the column of produced gas was 3 centimeters when measured shortly after isolation, and at a later date was only 0.5 centimeter long.[1]

The results of the sugar reactions were practically the same in all repeated tests. However, the production of gas was not uniform with the four cultures (G–1, G–2, G–3, and G–4). It also varied with the particular carbohydrates, becoming less in the course of time. The resulting carbohydrate reactions were

[1] Philip. Journ. Sci. 33 (1927) Table 1.

checked by titrating the acidity against N/20 sodium hydroxide (Table 4).

TABLE 4.—*Showing the results of titration after one week incubation.*

Strain.	Glucose.		Maltose.		Mannite.		Sorbite.	
	Infusion.	Peptone.	Infusion.	Peptone.	Infusion.	Peptone.	Infusion.	Peptone.
G–1	1.10	0.85						
G–2			1.35	0.90				
G–3					0 50	0.35		
G–4							1.30	0.75
G–5	1.00	0.75	1.20	0 80	1.20	0.65	1 20	0 70
Control	0.60	0.50	0.60	0.50	0.50	0.40	0 60	0 55

Strain.	Galactose.		Dulcite.		Saccharose.	
	Infusion.	Peptone.	Infusion.	Peptone.	Infusion.	Peptone.
G–1					0 85	0 70
G–2			1.10	0.75		
G–3	1.10	0 70				
G–4						
G–5	1.05	0.70	0 40	0 35	2 00	1.20
Control	0.85	0.50	0.50	0.40	0 90	0.70

SEROLOGY

Homologous sera were produced in rabbits with each of the five strains. With these sera cross-agglutination reactions were performed the results of which are recorded in Table 5. It shows that there are two serologically distinct types. The first four strains (G–1, G–2, G–3, and G–4) form a separate type; and the fifth strain (G–5) forms by itself another type. Absorption tests were also performed with the strains G–1, G–2, G–3, and G–4, using their respective homologous sera. Each strain absorbed its own agglutinins completely (Table 6). No absorption test was made with strain G–5.

An antihæmorrhagic cattle septicæmia serum [5] was obtained, and agglutination reactions were performed with the strains G–1, G–2, G–3, G–4, and G–5. As may be seen in Table 7 there was a low-grade but marked agglutination reaction noticeable with strain G–5.

[5] Dr. R. Gonzales, an agent of the Jensen Salsbery Laboratory, United States of America, kindly supplied this serum.

TABLE 5.—*Showing the results of cross agglutination reactions.*

Strain.	Serum G-1.				Serum G-2.				Serum G-3.			
	1 : 100	1 : 200	1 : 400	1 : 800	1 : 100	1 : 200	1 : 400	1 : 800	1 : 100	1 : 200	1 : 400	1 : 800
G-1	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
G-2	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
G-3	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
G-4	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
G-5	—	—	—	—	—	—	—	—	—	—	—	—

Strain.	Serum G-4.				Serum G-5.				Control, salt solution.
	1 : 100	1 : 200	1 : 400	1 : 800	1 : 100	1 : 200	1 : 400	1 : 800	
G-1	++++	++++	++++	++++	—	—	—	—	—
G-2	++++	++++	++++	++++	—	—	—	—	—
G-3	++++	++++	++++	++++	—	—	—	—	—
G-4	++++	++++	++++	++++	—	—	—	—	—
G-5	—	—	—	—	++++	++++	++++	++++	—

TABLE 6.—*Showing the results of absorbed sera by their homologous antigens with unabsorbed sera as controls.*

Strain.	Absorbed and unabsorbed sera.					
	G-1			G-2		
	1 : 100	1 : 200	Control 1 : 200	1 : 100	1 : 200	Control 1 : 200
G-1	—	—	++++	—	—	++++
G-2	—	—	++++	—	—	++++
G-3	—	—	++++	—	—	++++
G-4	—	—	++++	—	—	++++

Strain.	Absorbed and unabsorbed sera.					
	G-3			G-4		
	1 : 100	1 : 200	Control 1 : 200	1 : 100	1 : 200	Control 1 : 200
G-1	—	—	++++	—	—	++++
G-2	±	—	++++	—	—	++++
G-3	—	—	++++	—	—	++++
G-4	—	—	++++	—	—	++++

TABLE 7.—*Showing the result of agglutination test of the five strains against the antihæmorrhagic septicæmia serum (for cattle).*

[Jensen Salsbery Laboratory, U. S. A.]

Polyvalent serum dilution.	G-1	G-2	G-3	G-4	G-5
1 : 25	—	—	—	±	++++
1 : 50	—	—	—	—	+++
1 : 100	—	—	—	—	+++
1 : 200	—	—	—	—	+
1 : 400	—	—	—	—	±
1 : 800	—	—	—	—	—
Control	—	—	—	—	—

COMMENTS

The gas-forming organisms hitherto studied and described were found to give biochemical reactions distinct from those of the ordinary non-gas-forming organisms. Their morphology, cultural characteristics, and behavior with regard to the production of agglutinins approximate those of the group of non-gas-forming organisms. The non-gas-forming bacteria, described in this paper, were undoubtedly *B. bovisepticus*.

In immunizing rabbits about five slants were needed to produce a titer of 1 to 800 for over a period of forty-six days. With strain G–5 a serum of a higher titer (1 to 3,200) was obtained, using the same amount of antigen for the same length of time as was required for the other strains.

One rabbit, which received one slant of killed culture over a period of fourteen days, revealed no agglutinins in its blood in a dilution of 1 to 50. Other rabbits, which received intravenously for over forty-six days 1½ slants heated to 56° C., did not show the presence of agglutinins in the dilution of 1 to 50; but a single injection of three-fourths of a slant of living culture produced immediately a rapid increase of agglutinins in a dilution of 1 to 640 in the same rabbit (Table 8).

As noted by L. M. Roderick,[6] L. Barnes,[7] and others, the immunity might set in before the antibodies were demonstrable in the blood. The process of immunization may be further advanced with the use of living organisms.

TABLE 8.—*Showing the procedure of immunization to produce agglutinating sera in rabbits.*

Strain.	Vaccine heated to—	Injection.	Amount (slants) injected.	Days of immunization.	Titer of serum.
	°C.				
G–1	56	Intravenous	1	14	1 : 50 = 0
	56	do	3	23	1 : 200
	50	do	[a]1	13	1 : 800
G–2	56	do	2.75	23	1 : 200
	50	do	[a]1	13	1 : 800
G–3	56	do	1	14	1 : 50 = 0
	56	do	3	23	1 : 200
	50	do	[a]1	13	1 : 800
G–4	56	do	1	14	1 : 50 = 0
	56	do	3	23	1 : 200
	50	do	[a]1	13	1 : 800
G–5	56	do	4.5	46	1 : 3200
G–1	56	Intravenous	1.75	46	1 : 50 = 0
	(b)	Subcutaneous	[a]0.75	15	1 : 640
G–4	56	Intravenous	1.75	46	1 : 50 = 0
	(b)	Subcutaneous	[a]0.75	15	1 : 640

[a] Once. [b] Alive.

The difficulty of producing serum of high agglutinating titer in a relatively short time with most of the hæmorrhagic septi-

[6] Journ. Infect. Dis. 31 (1922) 313-325.
[7] Journ. Immunology 15 (1928) 289-297.

cæmia strains has been experienced by other workers. A. Tanabe,[8] working with various strains of hæmorrhagic septicæmia organisms, obtained an agglutination titer of 1 to 640.

G. E. Jorgenson [9] obtained a titer of 1 to 500 after the injection of killed cultures over a period of thirty-one days, but a clear cut agglutination was not so prompt as is usual with other bacteria. Further treatment with killed cultures to increase the titer of this serum to 1 to 5,000 was possible according to him.

The above observations were also noted in my experimental animals, and the absorption reaction was not difficult, being applied once only.

It is known from the study made by V. A. Moore [10] that the hæmorrhagic septicæmia organisms are found in the air passages of apparently healthy animals in the proportion of about 80 per cent in cattle; and about 48 per cent in hogs that were examined by him. Lately G. E. Jorgenson [11] resumed the study of hæmorrhagic septicæmia organisms from two hundred fifty normal cattle of which thirty-seven animals harbored in their nasal passages the *Pasteurella* organisms.

The gas-forming bipolar organisms were also encountered in some animals with symptoms of hæmorrhagic septicæmia. However, very little study was made in this direction. The finding of the two distinct types, gas-forming and non-gas-forming bacteria, in the deeper organs such as lymphatic glands suggests that both types may, independently, produce the same type of disease, attributed solely to non-gas-forming hæmorrhagic septicæmia organisms. However, this concept requires further analysis of cases studied by experimental procedures.

The finding of a hæmorrhagic septicæmia organism in the lymphatic glands indicates its natural route. The blood stream is invaded in due time when the resistance of the animal becomes lowered. The disease has a rapid course. "Some animals, which are apparently healthy, will turn around once or twice and drop dead; or, if tied by halter will apparently try to break loose from their manger and drop dead." [12]

[8] Journ. Infect. Dis. 33 (1926) 241–248.
[9] Cornell Veterinarian 15 (1925) 295–302.
[10] U. S. Dept. Agr. Bur. of Animal Industry, Bull. 3.
[11] Cornell Veterinarian 15 (1925) 295–302.
[12] Philip. Agr. Rev. 1 (1908) 135.

SUMMARY AND CONCLUSIONS

1. Both gas-forming and non-gas-forming bipolar organisms were isolated from the lymphatic adenitis of slaughtered cattle. They are morphologically alike.

2. The gas-forming organism fermented glucose, galactose, levulose, maltose, sorbitol, dulcitol, inulin, and mannose. The non-gas-forming organism fermented glucose, galactose, levulose, maltose, sucrose, sorbitol, mannitol, inulin, and mannose. Therefore, the organism was identified as *B. bovisepticus*. Neither type grew on eosin-methylene blue-lactose agar. Each type gave definite and distinct biochemical and serological reactions of its own.

3. Both types were pathogenic for rabbits and guinea pigs.

4. Some immunological phenomena, like the early appearance of immunity before antibodies are demonstrable commonly noted by some workers with hæmorrhagic septicæmia organisms, were likewise observed in the animals injected with our gas-forming bacteria.

ACKNOWLEDGMENT

I wish to express my most sincere thanks and appreciation to Dr. R. B. de Leon, of the Philippine Health Service, for supplying the materials of study, and to Dr. Otto Schöbl, chief of the division of biology and serum laboratory, Bureau of Science, for his kind assistance.

CULTIVATION OF AN ACID-FAST BACILLUS FROM LEPROSY

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ONE PLATE

The combat against leprosy would be greatly aided by the cultivation of Hansen's bacillus, and by the discovery of preparations of the antigen suitable for determining susceptibility to, and creating immunity against, the disease. The exacerbations in leprosy are characterized by marked hypersensitivity and it might well be that desensitization would contribute to its therapy.

When cultivating bacteria one must furnish suitable respiratory conditions as well as proper food. Since the writer and Ervin [1] had shown that carbon dioxide (CO_2) was essential for the growth of *B. tuberculosis*, and since Rockwell [2] had demonstrated the same fact for all of a number of other bacteria, special attention was given to this fact in the attempt to cultivate Hansen's bacillus. Variations in the oxygen (O_2) and carbon dioxide (CO_2) supply were brought about as follows:

(*a*) *Aërobic.*—The culture tubes were left uncapped, or when covered by a rubber cap, a fine syringe needle inserted through the cap allowed air to enter.

(*b*) *Little oxygen* (O_2) *and increased amount of carbon dioxide* (CO_2).—The culture tubes were attached by means of gum rubber tubing to agar slants which had been inoculated with *B. coli.*

(*c*) *Oxygen* (O_2) *and carbon dioxide* (CO_2).—The tubes were prepared as in (*b*) and then a fine syringe needle was inserted through the connecting rubber tubing and the point of the needle buried in the cotton plug of the culture tube.

(*d*) *No oxygen* (O_2) *but carbon dioxide* (CO_2) *present.*—The anaërobic condition was brought about by Rockwell's method (pyrogallic acid and sodium bicarbonate).

[1] Journ. Infec. Dis. 22 (1918) 194.
[2] Journ. Infec. Dis. 32 (1923) 93.

CULTIVATION EXPERIMENTS

Several native fruits and vegetables autoclaved in 3 per cent glycerin solution were used. These were the mango, papaya, lansone, bean, opo, yellow squash, its seeds, ampalaya, taro, eggplant, and togue (germinated mongo beans). When planted upon or in these media, the lepra bacilli were recovered in smears a few days after inoculation and for as long as seven to fourteen days in some instances, but there were no signs that the bacilli had multiplied. Sometimes intact nucleated lepra cells containing stainable nuclei and numerous acid fasts were seen. There was no proliferation on 3 to 6 per cent glycerin agar or glycerin agar containing human blood.

The growth described below was obtained in a modification of glycerin agar containing hen's ovomucoid and yolk. The preparation of this is as follows:

The white of a hen's egg is boiled in 100 cubic centimeters of distilled water containing 3 to 6 per cent glycerin. This is filtered through cotton. About one-half of the yolk of the egg is thoroughly mixed with the filtrate and boiled. This is filtered through gauze in order to allow the finer yolk particles to pass the filter. This is then autoclaved at 20 pounds for twenty minutes. In the following experiments the glycerinized ovomucoid yolk and nutrient agar containing 2 per cent of agar were mixed aseptically in equal proportions. This medium is semisolid. The planted lepra bacilli tend to persist for several weeks upon this medium, and in one instance where this medium contained 1 to 10,000 gentian violet, the acid-fast bacilli persisted without apparent multiplication for more than three months under respiratory conditions (*b*) and (*d*).

In the attempt to stimulate growth, small amounts of various substances were added; for example, potassium dihydro-phosphate (KH_2PO_4), sodium hydrogen phosphate (NA_2HPO_4), calcium chloride ($CaCl_2$), magnesium nitrate ($MgNO_3$), potassium iodide (KI); and glucose, lactose, saccharose, maltose, mannite; and oleic, palmitic, butyric acids; and peanut oil and lumbang oil.

In most instances these combined media were prepared and used as follows: The autoclaved ingredients were placed in small sterile plugged tubes and mixed by drawing back and forth in a sterile pipette, solidified in a slant position and kept on ice for twenty-four hours in order to allow water of syneresis to col-

lect. As a general rule, 2 to 4 cubic centimeters of media were used.

In most of the experiments the planted bacilli disappeared in a few days, or without signs of multiplication, persisted for one or two weeks. The only combination tried in which multiplication took place was when 1 or 2 drops of autoclaved oleic acid and 1 or 2 drops of a 10 per cent dextrose autoclaved in distilled water were added to each cubic centimeter of the glycerinized agar ovomucoid yolk medium.

I am indebted to Dr. E. V. Pineda, of San Lazaro Hospital, for his assistance in making the cultures. Recently discovered cases that had not been treated were chosen. The skin over the leprous lesion was cleaned with iodine and alcohol, and blood containing lepra bacilli was obtained on the edge of a sterile knife by the routine "snip" method. One loopful of blood was then transferred by means of a sterile platinum loop to the water of syneresis in the culture tubes. Control smears showed that numerous lepra bacilli were always transferred in the loopful.

None of the cultures was contaminated by cocci or diphtheroids from the skin. Cultures from three cases of leprosy, kept at 35° to 37° C. showed decided proliferation at the end of four to six weeks. The best growth was obtained in cultures that were kept first at partial oxygen tension [little oxygen (O_2) but carbon dioxide (CO_2) present] for a month, after which the tubes were kept under oxygen (O_2) and carbon dioxide (CO_2). Proliferation is recognized only by making smears of the semisolid culture medium. It is important that the contrast stain, Löffler's methylene blue, should be diluted for a too dense staining of the medium will obscure many of the fine acid-fast rods. The nuclei of planted lepra cells disappeared and the microscopic colonylike masses of acid-fast rods increased in number for a few weeks and then the growth appeared to be stationary (Plate 1).

Subculture of a loopful of material containing several dozen colonies into the same medium resulted in the appearance of a large number of subcolonies and isolated masses and scattered acid fasts. The subcultures were examined five weeks after inoculation. Two of the primary cultures in the above medium were subcultured successfully in the same medium, but the transplanted bacilli disappeared when they were carried over into various other modifications of the glycerinized ovomucoid yolk

medium. In one instance, the primary culture was three and a half months old and, in another instance, one month old when the subcultures were made.

The rods are thinner than tubercle bacilli, and when Löffler's blue is used as a contrast stain, they often contain one or two blue granules. As in the case of smears from leprosy, if the culture preparations are first treated with xylol and alcohol, the rods do not retain the stain after heating with carbol fuchsin and treating with acid alcohol. This peculiarity of lepra bacilli in smears has been noted also by Dr. E. V. Pineda.

SUMMARY

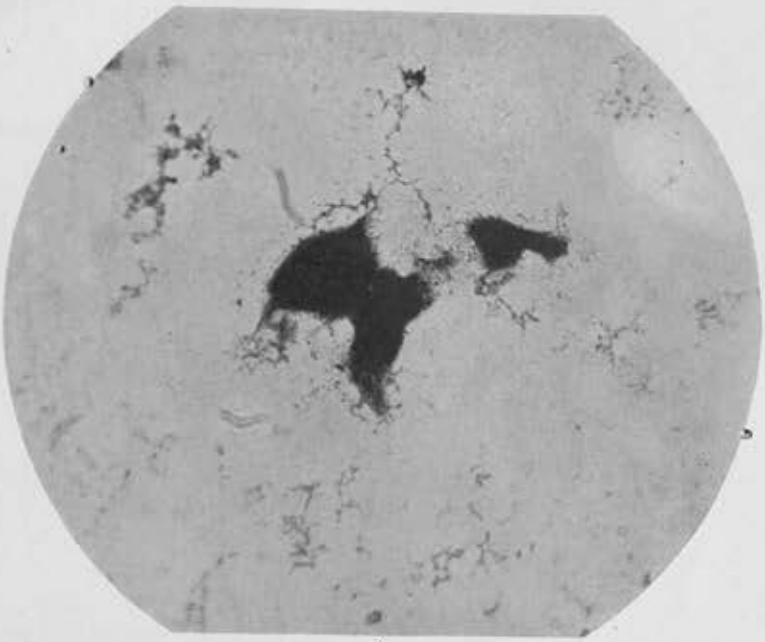
An acid-fast bacillus was grown in a semisolid medium composed of equal parts of nutrient agar, and hen's ovomucoid and yolk, prepared by boiling in 3 to 6 per cent glycerin solution, when there was added to this 1 or 2 drops of oleic acid and 1 or 2 drops of 10 per cent dextrose solution for each cubic centimeter of medium. Multiplication occurred best in cultures incubated at 35 to 37° C., with little oxygen present but carbon dioxide present. Microscopic examination after four to six weeks incubation showed the presence of numerous microscopic colonies. Growth was obtained from three cases. From two of the primary cultures, subcultures were obtained at a time when the primary cultures were three and one-half months and one month old, respectively. Like the lepra bacilli in smears from cases, the cultivated bacilli do not stain by the acid-fast method if they are first treated with xylol and alcohol.

ILLUSTRATION

PLATE 1

FIG. 1. One of the larger microscopic colonies. Isolated minute acid-fast bacilli scattered about the neighborhood.

2. Younger, more loosely aggregated masses of growth.



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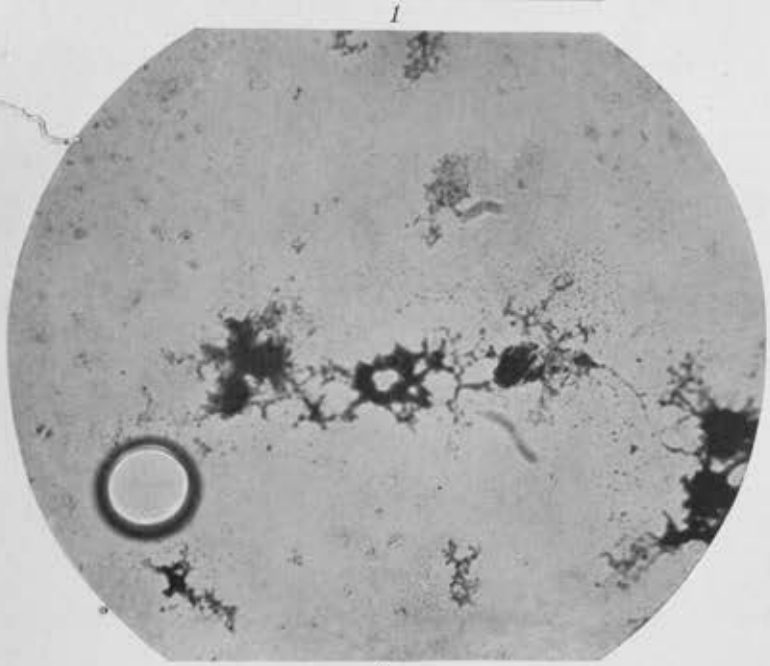

2

PLATE 1.

IMMUNOLOGIC RECIPROCITY BETWEEN SYPHILIS AND YAWS[1]

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The effect of infection with syphilis on the development of immunity to yaws was studied in two experiments.

The first experiment was arranged in the same way as were those of the early workers who studied the cross immunity between syphilis and yaws in monkeys. (Neisser, Baermann, Halberstaedter, Castellani, Levaditi, and Larier.)

Monkeys were inoculated with syphilis by intradermal injection of syphilitic material on the scrotum. At intervals of time after this inoculation the animals were inoculated with yaws by intradermal injection in the eyebrows. The intervals of time between the two inoculations ranged from two and one-fourth months to eight months. Every one of the monkeys inoculated with syphilis and giving evidence of syphilitic infection, either by specific lesion, by presence of viable treponemas in the lymph glands, or both, developed yaws at the point of yaws inoculation, which inoculation was performed within seven months after the inoculation with syphilis. The monkey that was successfully inoculated with syphilis and superinoculated with yaws eight months later failed to show yaws lesion on two successive inoculations.

In the second experiment the animals were inoculated with syphilis repeatedly. The subsequent inoculations failed to produce lesions in the syphilitic monkeys, while normal control monkeys showed a typical specific lesion at the place of inoculation with syphilis. The inocula, therefore, contained viable treponemas but the syphilitic monkeys failed to react by specific lesions to the superinoculations with syphilis. Thus it was proven that the animals became immune to syphilis. Following the superinoculation with syphilis the monkeys were

[1] Presented for publication June 4, 1930.

inoculated repeatedly with yaws on the eyebrows, while the previous inoculations with syphilis were performed on the scrotal skin. Normal monkeys were inoculated at the same time with the same amount of the same yaws material and served as controls (see Table 2). The result of the second experiment confirms the findings made in the first one that infection with syphilis confers an immunity to yaws in Philippine monkeys. This cross immunity develops much later than immunity to syphilis in syphilitic monkeys and later than yaws immunity in yaws monkeys.

DISCUSSION

The first and the second experiments give us a good deal of the desired information. They show first of all that Philippine monkeys become immune to syphilis in less than two months after the first intracutaneous inoculation with syphilis; in other words, about as soon as rabbits do. Philippine monkeys inoculated with syphilis become immune to yaws later than yaws monkeys become immune to yaws.

The second experiment shows that intradermal superinfection with yaws of syphilitic monkeys performed after the animals become imune to syphilis but still are susceptible to yaws does not hasten the immunity to yaws. This is naturally to be expected since intradermal superinoculation with yaws of yaws monkeys does not hasten the immunity to yaws within the six months period. The second experiment also demonstrates that superinoculation with syphilis of syphilitic animals, performed in the stage of tissue nonreactivity with regard to syphilis, does not influence the onset of immunity to yaws.

In these experiments the fallacy of the theory of latent infection as a cause of resistance to inoculation in syphilis is again evident. Syphilitic monkeys with syphilitic "latent infection" were not immune to yaws at a time when yaws monkeys without "latent infection", due to either yaws or syphilis, were found on a previous occasion immune to both yaws and syphilis. That this theory is untenable has been pointed out by several workers in the literature. We believe that the results of our immunologic experiments are more convincing of the fallacy of this theory than any other experimental evidence presented before in favor or disfavor of this most unique of all the theore-

tical explanations of immunity that have ever been offered in medicine.

The findings that monkeys infected with syphilis became immune to syphilis much sooner than they did to yaws and that monkeys infected with yaws became immune to yaws sooner than to syphilis prove that the immunologic reciprocity between yaws and syphilis is a group immunity quite similar to group relationship known to exist in bacterial immunity.

Differences may exist between individual strains of treponema of syphilis with regard to cross immunity of heterologous strains, but the significance of these differences appears to have been unduly exaggerated in the literature. No rational explanation of these immunologic peculiarities of individual treponema strains of the same kind is possible as long as the theory of latent infection is accepted as an explanation of resistance to inoculation and the existence of immunity to syphilis is denied. Whatever these variations may be, it cannot be denied that treponema of syphilis and treponema of yaws represent the utmost extremes in this group of parasites. Consequently the immunologic reciprocity between these two proves the existence of pantreponematous immunity as the highest degree of group immunity.

CONCLUSIONS

1. Immunologic reciprocity exists, not only between yaws and syphilis but also between syphilis and yaws.

2. Syphilis in Philippine monkeys produces immunity to itself much sooner than it does to yaws and sooner than yaws does to itself.

3. Superinoculation with syphilis of syphilitic monkeys performed in the stage of tissue nonreactivity does not hasten the immunity to yaws.

4. Superinoculation of syphilitic monkeys with yaws, performed at a time when they have reached the stage of nonreactivity to syphilis but still react by formation of typical yaw to inoculation with yaws, does not hasten the immunity to yaws within the period tested.

5. The immunologic reciprocity between yaws and syphilis is group immunity. One of the two immunizes against itself quicker than against the other.

TABLE 1.—*Showing the results of cross inoculation with yaws of monkeys that received a single intradermal inoculation with syphilis.*

(+, positive take; —, negative take; 0, not done; D, died. The animals were observed for six months after the last inoculation.)

Designation of monkey.	Inoculated with syphilis.		Lymph-gland transplants (syphilis).		Inoculated with yaws.	
	Date.	Result.	Date.	Result.	Date.	Result.
Sy-E-42	[a]X-27-28	+	0	0	I- 7-29	+
Sy-6	VI-15-28	+	X-22-28	+	IX-11-28	+
Sy-3	VI-13-28	+	IX- 8-28	+	IX-11-28	+
Sy-1	V-16-28	+	IX- 8-28	+	IX-11-28	+
Sy-5	VI-15-28	+	X-22-28	+	XI-13-28	+
Sy-J-20	II- 9-29	+	0	.....	X- 8-29	—
					XII- 2-29	...
Two controls	0	.....	0	.....	X- 8-29	+
						+
Do	0	.....	0	.....	XII- 2-29	+
						+

[a] These letters and figures indicate month, day, and year; thus, X-27-28 means October 27, 1928.

TABLE 2.—*Showing the results of experiments concerning immunologic reciprocity between syphilis and yaws.*

[+, positive take; —, negative take; 0, not done; D, died. The animals were observed for six months after the last inoculation.]

Designation of monkey.	Intradermal inoculation with syphilis.					
	Date.	Result.	Date.	Result.	Date.	Result.
Sy-G-20	II- 5-29	+	V-29-29	—	IX-27-29	—
Sy-J-21	II- 9-29	+	V-29-29	—	IX-27 29	—
Sy-J-20	II- 9 29	+	0		0	
Sy-I-11	III-20-29	+	VIII-14-29	—	0	
Sy-I-12	III-20-29	+	VIII-14-29	—	0	
Sy-G-22	VI-22-29	+	VIII-14-29	—	0	
Sy-G-23	VI-22-29	+	VIII-14-29	—	0	
Sy-P-23, control	0		VIII-14-29	+	IX-27-29	—
Sy-P-24, control	0		0		IX-27-29	+
Sy-P-25, control	0		0		IX-27-29	+
Y-G-24, control	0		0		0	
Y-G-25, control	0		0		0	
YM-20, control	0		0		0	
YM-21, control	0		0		0	
YM-22, control	0		0		0	
YM-23, control	0		0		0	
Ya-C-14, control	0		0		0	
Ya-C-15, control	0		0		0	
Ya-C-16, control	0		0		0	

TABLE 2.—*Showing the results of experiments concerning immunologic reciprocity between syphilis and yaws*—Continued.

[+, positive take; —, negative take; 0, not done; D, died. The animals were observed for six months after the last inoculation.]

Designation of monkey.	Intradermal superinfection with yaws.							
	Date.	Result.	Date.	Result.	Date.	Result.	Date.	Result.
Sy-G-20	X- 8-29	—	XII- 2-29	—	0		0	
Sy-J-21	X- 8-29	—	XII- 8-29	D, XII- 9-29	0		0	
Sy-J-20	X- 8-29	—	XII- 2-29	—	0		0	
Sy-I-11	IX-21-29	+	X-21-29	+	I- 6-30	—	II-26-30	—
Sy-I-12	IX-21-29	+	X-21-29	+	I- 6-30	—	D, II-19-30	
Sy-G-22	X-14-29	+	X-21-29	+	I- 6-30	+	II-26-30	—
Sy-G-23	X-14-29	+	X-21-29	+	I- 6-30	D, I-21-30	0	
Sy-P-23, control	X-14-29	+	X-21-29	+	I- 6-30	+	II-26-30	—
Sy-P-24, control	0		0		0		0	
Sy-P-25, control	0		0		0		0	
Y-G-24, control	X- 8-29	+	0		0		0	
Y-G-25, control	X- 8-29	+	0		0		0	
YM-20, control	IX-21-29	+	X-21-29	+	0		0	
YM-21, control	IX-21-29	+	X-21-29	+	0		0	
YM-22, control	X-14-29	+	0		I- 6-30	+	D, I-25-30	
YM-23, control	X-14-29	+	0		0		II-26-30	D, III-4-30
Ya-C-14, control	0		XII- 2-29	+	0		0	
Ya-C-15, control	0		0		I- 6-30	+	II-26-30	+
Ya-C-16, control	0		0		I- 6-30	+	II-26-30	+

THE IMMUNOLOGIC EFFECT OF REPEATED YAWS INFECTIONS INTERRUPTED BY SPECIFIC TREATMENT GIVEN IN THE EARLY STAGE OF INITIAL YAW [1]

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The present experiment was planned in such a way that monkeys inoculated with yaws were treated with neosalvarsan before the third month after the inoculation; that is to say, before the incubation of the generalized manifestations expired. The object of this experiment was to find out whether or not this procedure of repeated inoculations with viable yaws material would produce immunity to yaws when each of the successive local infections were terminated by specific treatment before the time when generalized manifestations appeared.

In previous experiments it became known that Philippine monkeys, which had local yaws infection for three months or more, were found immune in the sixth or seventh month after the inoculation. When yaws monkeys were treated before the third month after the inoculation they were found susceptible to yaws in the seventh month.[2]

THE EXPERIMENT

The first monkey (m–5) was inoculated with yaws in October, 1928. After an incubation of thirty days a lesion developed that assumed the clinical character of a typical yaw forty days after inoculation. Treponemas were found by dark field in the scrapings made from this lesion. Neosalvarsan treatment was given on the fortieth day after inoculation. The lesion healed within a week.

This animal was again inoculated with yaws June 29, 1929; that is, eight months after the original inoculation with yaws.

[1] Presented for publication June 4, 1930.

[2] Philip. Journ. Sci. 35 (1928) 291.

The incubation of the lesion was long, but the lesion was a typical initial yaw and contained treponemas. Consequent to the long incubation, neosalvarsan treatment was not given until the seventy-second day after the second inoculation. The lesion healed rapidly, due to two injections of neosalvarsan. Thirteen days after the last therapeutic injection, and a little less than three months after the second inoculation, the animal was again inoculated; this time simultaneously with four other monkeys, which received a similar course of treatment. The monkey under discussion (m-5) failed to develop a lesion within a month, although its mates developed typical yaws within this period of time. Treatment was given five weeks after this unsuccessful inoculation, and the animal was inoculated the fourth time and developed typical yaw in four weeks. Three months and three weeks later the animal proved to be immune to inoculation with yaws.

In the rest of the animals included in this experiment a somewhat different plan was followed. The reinfections with yaws, interrupted by treatments, were fewer but were given at shorter intervals of time. They received fewer inoculations in rapid succession. Unfortunately, three of the four animals died after the last inoculation; one of them, however, lived long enough to show that the incubation became prolonged. The fourth monkey lived, and two successive tests for immunity were performed. This animal proved to be immune six months three weeks after the original inoculation with yaws.

DISCUSSION

In a previous experiment on six healthy volunteers[3] one behaved irregularly with regard to incubation, but the rest showed very regular incubation upon the first inoculation.

Volunteers A and B developed initial yaws in three and a half weeks; volunteers C, D, and F, four weeks after the inoculation. Volunteers A and B were superinoculated four weeks after the first inoculation, and the incubation of the initial yaw was again three and a half weeks. Volunteers C and D were superinoculated five weeks after the first inoculation and the incubation period was four and a half in one and six weeks in the other volunteer. Volunteer E was superinoculated six weeks after the first inoculation, and local yaw developed in five weeks after the superinoculation.

[3] Sellards, Lacy, and Schöbl, Philip. Journ. Sci. 30 (1926) 463.

All volunteers who were superinoculated more than four weeks after the first inoculation showed a longer period of incubation the second time than they did after the first inoculation. The generalization of the yaws process due to the first inoculation having appeared by that time, the volunteers were treated to the complete clinical and serologic cure and no further inoculation was performed.

In the present experiment on animals very similar conditions were encountered; that is, a prolongation of the incubation periods in the same animals that showed regular incubation in the early inoculations.

The animal (m–5) that received three interrupted inoculations within eleven months was not immune in thirteen months, but was found immune in seventeen months; while K–16 and, very likely, N–20 that received three interrupted inoculations within four months were immune in the seventh month after the original inoculation with yaws. These results agree with our early findings on experimental yaws that brought out the direct quantitative proportions between the severity of early yaws infection and the degree of subsequent immunity, as well as the inverse proportion between the severity of early infection and the time of onset of immunity. The more severe the early infection, the higher the degree of immunity and the earlier it sets in. These results, therefore, agree with the law of inverse proportions as formulated by Brown and Pearce for syphilis, which law in turn is supported by clinical experience. From the point of view of immunization the most opportune time is thereby indicated for the accomplishment of successful immunization. It is evident that the immunization in treponematoses must be fully accomplished during the period of full or even exaggerated tissue reactivity to the incorporation of treponema antigen. This view is further corroborated by our previous findings [4] that superinfection of yaws monkeys with syphilis performed in the early stage of yaws infection accelerated the immunity to yaws, while superinfection with syphilis of syphilitic monkeys performed in the stage of nonreactivity to syphilis had no appreciable accelerating effect on the onset of immunity to yaws.

CONCLUSIONS

1. Repeated local yaws infections terminated by specific treatment given before the time when generalized yaws manifesta-

[4] Philip. Journ. Sci. 42 (1930) 241.

TABLE 1.—*Showing the accumulative immunologic effect of repeated infections interrupted by specific treatment in the early period of yaws infection.*

[+, positive take; —, no take; ?, no take up to the time of treatment; 0, not done; D, died.]

Designation of monkey.	Inoculation.			Neosalvarsan.		Inoculation.			Neosalvarsan.	
	Date.	Result.	Incubation, weeks.	Date.	Gram.	Date.	Result.	Incubation, weeks.	Date.	Gram.
m-5	*X-20-28	+	4	XII- 1-28	0.02	VI-29-29	+	6	IX- 1-29	0.0115
									IX- 5-29	0.0115
K-16	VIII- 1-29	+	4	IX- 3-29	0.01125	IX-18-29	+	4	X-24-29	0.0115
				IX-10-29	0.01125				X-29-29	0.0115
K-17	VIII- 1-29	+	4	IX- 3-29	0.01125	IX-18-29	+	4	X-24-29	0.0115
				IX-10-29	0.01125				X-29-29	0.0115
N-20	VIII- 1-29	+	4	IX- 3-29	0.01125	IX-18-29	+	4	X-24-29	0.0115
				IX-10-29	0.01125				X-29-29	0.0115
N-21	VIII- 1-29	+	4	IX- 3-29	0.01125	IX-18-29	+	4	X-24-29	0.0115
				IX-10-29	0.01125				X-29-29	0.0115
Yac-20, control	0			0		0			0	
Do	0			0		0			0	
Ya-C-27, control	0			0		0			0	

Designation of monkey.	Inoculation.			Neosalvarsan.		Inoculation.			Inoculation.			Confirmatory immunity test.	
	Date.	Result.	Incubation, weeks.	Date.	Gram.	Date.	Result.	Incubation, weeks.	Date.	Result.	Incubation, weeks.	Date.	Result.
m-5	IX-18-29	?		X-24-29	0.016	XI-26-29	+	4	III-15-30	—		V-20-30	—
K-16	XI-26-29	+	4	I-21-30	0.015	0			II-18-30	—		V-20-30	—
K-17	XI-26-29	+	4	I-21-30	0.015	0			II-18-30	?	D, II-25-30	0	
N-20	XI-26-29	+	4	I-21-30	0.015	0			II-18-30	—	D, III-25-30	0	
N-21	XI-26-29	?		I-21-30	0.015	0			II-18-30	?	D, II-25-30	0	
Yae-26, control	0			0		0			II-18-30	+	4	0	
Do	0			0		0			III-15-30	+	4	0	
Ya-C-27, control	0			0		0			0			V-20-30	+

[a] These letters and figures indicate month, day, and year; thus, X-20-28 means October 20, 1928.

tions occur produce immunity in Philippine monkeys, even though delayed.

2. The incubation period becomes irregular, mostly prolonged with the repeated reinoculations terminated by treatments. This shows that the developing immunity, although not yet strong enough to suppress completely the development of lesion, has a definite effect upon the incubation period. This observation strengthens our claim that sensitization is the underlying principle of immunity in treponematoses.

3. The findings made on monkeys corroborate and amplify the findings previously made on human volunteers and prove that immunization against yaws by means of repeated local yaws infections terminated by treatments is possible.

4. Jointly with our previous experimental evidence these results indicate that the most favorable time for immunization in treponematoses is the early stage of infection and that it must be carried out vigorously within the first few months of infection or immunization.

THE DURATION OF ANTITREPONEMATOUS IMMUNITY WITH REGARD TO SYPHILIS IN PHILIPPINE MONKEYS

By Otto Schöbl

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In the course of our investigation concerning cross immunity between the two human treponematoses, yaws and syphilis, experimental animals have accumulated that have been inoculated at various times with yaws, with syphilis, or both. In order to utilize these experimental animals to the full extent they were employed in the present experiment. The same animals having been used in previous experiments, the dates concerning the onset of immunity are necessarily given here again. There are two groups of animals represented in Table 1.

The first group contains animals that were inoculated first with yaws, then repeatedly proven immune to yaws and to syphilis. They were tested for immunity to syphilis by intracutaneous inoculation with syphilis (Nichols strain), two and one-half, three and one-half, three and five-sixths, and four and three-fourths years after the original inoculation with yaws. They failed to develop lesions at the place of inoculation. Normal control monkeys inoculated at the same time with the same amount of the same syphilitic material developed typical scleroses.

In the second group were placed monkeys that were originally inoculated with syphilis by intradermal injection. They were then superinoculated with yaws; some while still susceptible, others when already immune to yaws. The final test for immunity to syphilis was performed and properly controlled three, seven, eight, eleven, and nineteen months after the original inoculation with syphilis. They were found immune to intradermal inoculation with syphilis.

TABLE 1.—*Showing the results of experiments concerning the duration of immunity to syphilis produced either by yaws or syphilis infection in Philippine monkeys.*

[+, typical take; —, no take; ±, immune reaction; 0, not done; D, died.]

Designation of monkey.	Inoculated with yaws.		Inoculated with syphilis.		Lymph-gland transplants.		Rabbits.		Test for immunity to syphilis.	
	Date.	Result.	Date.	Result.	Date.	Result.	Lived, V-15-29.	Died.	Date.	Result.
D-18	*IV-25-25	+	VI-15-28	±	XI-12-28	—	Two	—	I- 7-30	—
	III-15-26	—								
	V-14-26	—								
	IV- 4-27	—								
	II- 4-28	—								
	I-11-29	—								
J-11	III-19-26	+	IX-13-28	±	XI-16-28	—	One	One II-11-28	I- 7-30	—
	VII- 6-26	+								
	IX-29-26	—								
	IV- 4-27	—								
	III-26-28	—								
	I-11-29	—								
T-4	VII- 2-26	+	IX-13-28	—	XI-16-28	—	—	Two XII- 8-28 XII-10-28	I- 7-30	—
	VIII-20-26	+								
	III- 8-27	—								
	III-26-28	—								
	I- 6-29	—								
o-c	VII-27-26	+	IX-13-28	±	XI-16-28	—	—	Two XII-10-28 XII-12-28	I- 7-30	—
	IV- 4-27	—								
	III-26-28	—								
	I- 6-29	—								

Designation of monkey.	Inoculated with syphilis.		Inoculated with yaws.		Lymph-gland transplants.		Rabbits.		Test for immunity to syphilis.	
	Date.	Result.	Date.	Result.	Date.	Result.	Lived.	Died.	Date.	Result.
Sy-2	V-24-28	(?)	XI-13-28	+	X-22-28	+	One	—	I- 7-30	D
Sy-3	VI-13-28	+	IX-11-28	+	VI-26-28	+	One	—	I- 7-30	—
Sy-5	VI-15-28	+	XI-13-28	+	X-22-28	+	One	—	I- 7-30	—
Sy-G-20	I-29-29	+	X- 8-29	—	0				IX-27-29	—
	V-25-29	—	XII- 2-29	—						
Sy-J-20	II- 9-29	+	X- 8-29	—	0				I- 7-30	—
			XII- 2-29	—						
Sy-J-21	II- 9-29	+	X-8-29	—	0				IX-27-29	—
	V-29-28	—	XII- 2 29	D						
Sy-G-22	VI-22-29	+	X-14-29		0				IX-27-29	—
	VIII-14-29	—	X-21-29	+						
			XII- 2-29	+						
Sy-G-23	VI-22-29	+	X-14-29		0				IX-27-29	—
	VIII-14-29	—	X-21-29	+						
			XII- 2-29	+						
Sy-P-23, control	VIII-14-29	+								
Sy-P-24, control	0		0		0				IX-27-29	+
Sy-P-25, control	0		0		0				IX-27-29	+
Sy-8, control	0		0		0				I- 7-30	+
Sy-7, control	0		0		0				I- 7-30	+

[a] These letters and figures indicate month, day, and year; thus, IV-25-25 means April 25, 1925.

CONCLUSION

If it is considered that the immunity to syphilis in Philippine monkeys sets in about one and one-half months after the inoculation with syphilis and in about eight months after inoculation with yaws, the persistence of immunity to syphilis in Philippine monkeys is clearly evident. There is every reason to believe that immunity to syphilis, like immunity to yaws, lasts throughout the natural life of these animals.

THE DURATION OF ANTITREPONEMATOUS IMMUNITY IN PHILIPPINE MONKEYS ORIGINALLY CONVEYED BY IMMUNIZATION WITH KILLED YAWS VACCINE

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The purpose of this experiment was to ascertain the duration of immunity to yaws in animals that have been immunized with killed yaws vaccine and stood the test for immunity repeatedly, both to yaws and syphilis.[1]

Four animals survived the previous experiment up to date. They had been vaccinated in the latter part of 1927 and the early part of 1928. The ultimate test for the duration of immunity was performed in February, 1930. This test consisted of intradermal inoculation with viable yaws material. The viability of the inoculum was tested simultaneously on one normal control Philippine monkey. The interval of time between the first vaccination and the last test for immunity to yaws varies, therefore, from two to two and a half years. Following the vaccination and the early test for immunity to yaws by inoculation, the animals were tested for immunity to syphilis by inoculation with live syphilitic material. They were found immune to syphilis as far as the skin is concerned, although the treponemas of syphilis were found viable in the lymph glands corresponding to the point of inoculation with syphilis.

The immunity, the duration of which was tested in this experiment, is not the result of vaccination alone. The animals were inoculated with yaws and syphilis subsequent to the vaccination. Some of them, however, showed no specific lesion from the time of vaccination up to the last test for immunity. The condition with regard to infection was, therefore, different from the condition in the inoculated monkeys that developed

[1] Philip. Journ. Sci. 42 (1930) 219.

lesion and in which the duration of immunity was previously tested.[2]

There is little doubt that inoculation following vaccination increases the antitreponematous immunity. It is known that inoculation with yaws performed on vaccinated animals raises the serologic reactions in experimental animals.[3]

The close relation between the serologic response to infection and the onset of immunity has likewise been demonstrated.[4]

Furthermore, it has become known that superinfection with syphilis performed on yaws-infected monkeys, in the stage of tissue reactivity to syphilis, accelerates the development of immunity to yaws.[5]

On the other hand superinfection with syphilis performed on syphilitic monkeys in the stage in which the skin no longer reacts to the inoculation did not accelerate noticeably the immunity to yaws.[6]

It was, therefore, important to ascertain the duration of the antitreponematous immunity acquired under these conditions. The attached table gives the results of this experiment and shows that the immunity is of long duration.

CONCLUSIONS

Antitreponematous immunity in vaccinated monkeys that have been proven immune to skin inoculation with yaws and syphilis is of as long duration as the immunity induced by infection accompanied by specific skin lesion.

[2] Philip. Journ. Sci. 40 (1929) 49.
[3] Philip. Journ. Sci. 40 (1929) 61.
[4] Philip. Journ. Sci. 42 (1930) 203.
[5] Ibidem 241.
[6] Antea 583.

TABLE 1.—*Showing the results of test for duration of antitreponematous immunity performed on yaws-vaccinated Philippine monkeys.*

[+, lesion; —, no lesion; 0, not done. All normal control animals employed in the tests for immunity to yaws or to syphilis developed typical lesions.]

Designation of monkey.	Date of vaccination.			Date of test for yaws immunity.			Result.	Date of test for syphilis immunity.	Result.		Date of last test for yaws immunity.	Result.
									Lesion.	Glands.		
U-1	*VII-27-27	VII-30-27	VIII-3-27	IX-17-27	III-9-28	IV-23-28	--	I-7-29	—	0	II-14-30	—
W-23	I-18-28	I-31-28	II-8-28	II-27-28	IV-23-28	VI-4-28	—	I-7-29	—	+	II-14-30	—
W-25	I-20-28	I-31-28	0	II-27-28	V-3-28	VI-25-28	—	I-7-29	—	+	II-14-30	--
W-27	I-20-28	I-31-28	0	II-27-28	V-3-28	VI-25-28	--	I-7-29	--	0	II-14-30	—
Control, Ya-C-19	0	0	0	0	0	0	----	0	----	----	II-14-30	+

*These letters and figures indicate month, day, and year; thus, VII-27-27 means July 27, 1927.

THE IMMUNOLOGIC EFFECT OF ANTITREPONEMATOUS VACCINE THERAPY ADMINISTERED AFTER SPECIFIC TREATMENT WHICH WAS GIVEN IN THE EARLY STAGE OF INITIAL LOCAL YAWS IN PHILIPPINE MONKEYS

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ONE TEXT FIGURE

In order to test the possibilities of the beneficial effect that vaccination may have on the course of treponematous infections, the following experiment was carried out.

Four Philippine monkeys were inoculated with yaws on the eyebrows by intradermal injection. All of these animals showed early serologic response following the inoculation with yaws (text fig. 1). Three monkeys developed typical clinical yaws containing treponemas. One of the four monkeys (K–27) failed to develop clinical yaws up to the time when treatment was given but showed, like others, distinct early serologic response to the inoculation. Due to the failure on the part of this animal to develop a clinical lesion within seven weeks after the first inoculation with yaws, this animal was kept as one of the non-vaccinated yaws-control animals.

The original inoculation with yaws of the four monkeys was performed November 27, 1929, and neosalvarsan treatment was given January 21, 1930, and again January 27, 1930, the total amount of the drug given to each monkey by intramuscular injection being 0.03 grams. The yaws lesions healed within a week after the first injection of neosalvarsan. Following this treatment, monkeys K–25 and K–26 were given three subcutaneous injections of syphilis vaccine, heated at 60° C. for one hour, on February 13, February 25, and March 4, 1930. The other two of the four monkeys, K–27 and K–28, received no vaccination and were kept as non-vaccinated yaws controls.

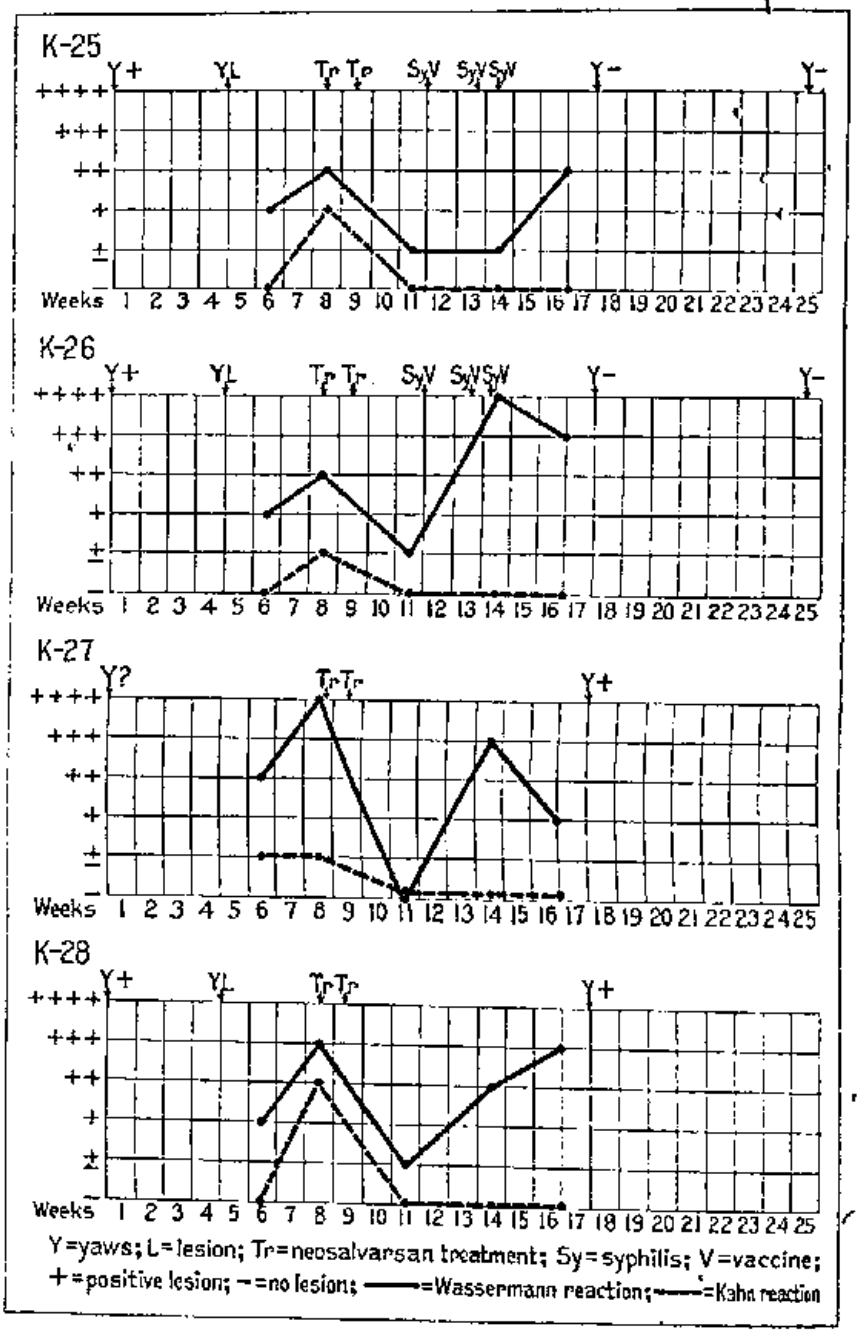

FIG. 1. Showing the serologic and immunologic effect of antitreponematous vaccine therapy given to yaws monkeys cured by neosalvarsan in the early stage of initial local yaws. The serologic reactions were kindly performed by Dr. Onofre Garcia, of the division of biology and serum laboratory, Bureau of Science, and were read jointly by him and the author.

The test for immunity was performed on these four monkeys by intradermal inoculation with viable yaws material on March 27, 1930; that is, four months after the original inoculation with yaws. Simultaneously with these monkeys a normal control monkey was inoculated in the same manner with the same amount of the same yaws inoculum.

Following the second inoculation with yaws as a test for immunity, the animals were inspected at frequent intervals and the following was found:

April 16, 1930, less than three weeks after the last inoculation, small oozing papular efflorescences were found at the places of inoculation on the eyebrows of the normal control monkey Ya–C–24 and the non-vaccinated yaws monkey K–28. These papules developed into typical yaws and contained treponemas.

April 23 a typical oozing papular lesion was found in the second non-vaccinated yaws monkey, K–27. This animal, it will be remembered, failed to develop a lesion upon first inoculation with yaws, within the time of seven weeks allowed to elapse between the inoculation and the specific treatment. Even though the yaws lesion that developed in this animal on the second inoculation healed rapidly, it had the typical clinical character and contained treponemas. Therefore, this animal was not immune at the time the second inoculation with yaws was performed as a test for immunity.

The two treated, and subsequently vaccinated, monkeys K–25 and K–26, showed a slight swelling at the place of the second inoculation in about two weeks, but no lesion developed. A confirmatory test for immunity was made on these two last-mentioned animals May 20, 1930. Simultaneously, one normal control monkey was inoculated with the same inoculum and in the same manner as the two vaccinated yaws monkeys. This test confirmed the previous test for immunity.

SUMMARY AND DISCUSSION

Four Philippine monkeys were inoculated with Kadangan strain of yaws and treated with neosalvarsan seven weeks after the inoculation; that is, in the early stage of the initial local yaw. Two of these four animals were vaccinated with killed syphilis vaccine (Nichols strain) subsequent to the chemotherapeutic cure. Test for immunity by inoculation with yaws, performed four months after the original inoculation, showed that the treated and vaccinated yaws monkeys were immune in four

months after the original inoculation, while the treated non-vaccinated yaws monkeys were not immune at that time. It is evident from this time relation that immunity in early treated and vaccinated yaws monkeys sets in just as soon after the vaccination as it does after generalization of the yaws process in untreated yaws infected animals.[1]

The great significance of the findings that vaccine treatment, administered to infected animals that were treated in the early stage of initial lesion, accelerates the onset of immunity will at once be realized when the law of inverse proportions, as formulated by Brown and Pearce for syphilis, is recalled to mind.

Although this law does not apply to yaws in the same manner that it applies in syphilis, it holds true in a general way. The more extensive and intensive the early yaws lesions, either initial or generalized, the less pronounced will be the late ulcerative or hyperkeratotic lesions. Monkeys with intensive exacerbations of initial yaws, followed by generalized eruptions, failed to develop late yaws lesions. On the other hand, animals that developed late lesions exhibited mild initial yaw and no generalized dissemination of lesions.

It became known from our early experiments on immunity to yaws that in animals with generalized yaws, so called secondaries, or in monkeys with late yaws lesions, so called tertiaries, no new lesions appeared once these animals became immune to inoculation. Thus, the effect of immunity upon the course of the infection was experimentally demonstrated. Furthermore, the case published from our institution by Miyao, indicates the effect of immunity on the course of yaws infection.[2]

A normal monkey was inoculated with Kadangan strain of yaws. It was treated with neosalvarsan less than three months after the inoculation. The lesions healed rapidly. One year after the treatment, the monkey was inoculated again with yaws. After long incubation an unusually late, fungoid, and ulcerative yaws lesion developed in this monkey. This shows that immunity was not fully developed in this animal at the time of reinfection with yaws and a late, so called tertiary lesion developed in consequence of reinfection.

[1] Philip. Journ. Sci. 35 (1928) 286.
[2] Philip. Journ. Sci. 41 (1930).

In the present experiment the early treated and vaccinated monkeys developed immunity in six weeks after the vaccination to such a degree that reinfection and consequently further stages of the infection were prevented. Thus, it was proven that antitreponematous vaccine therapy of an infected and early treated host has a beneficial effect on the course of the treponematous infection. The vaccine therapy under such conditions prevents not only reinfection of the host but also the occurrence of late lesions which are the most tragic features of treponematoses as illustrated in our early study on yaws in Philippine monkeys.[3]

CONCLUSIONS

1. Vaccine therapy with killed syphilis vaccine performed on yaws-infected and early treated monkeys accelerates the onset of immunity to yaws.

2. The immunity to yaws as a consequence of syphilis vaccine treatment administered to early cured yaws monkeys develops earlier than it does in early cured non-vaccinated yaws monkeys and earlier than it develops in untreated yaws monkeys with local yaws only.

3. The immunity under the conditions of vaccine treatment just mentioned, develops as early after the vaccination as it does in yaws-infected untreated monkeys after the generalization of the yaws process.

4. These findings are further proof of immunologic reciprocity between yaws and syphilis [4] and corroborate our previous findings that superinfection with syphilis of yaws-infected monkeys accelerates immunity to yaws.[5]

5. This experiment proves our contention deduced from former experiments [6] that vaccination administered early to recently sensitized animals has the same immunologic effect as generalization of the treponematous process. It confirms our former findings concerning vaccination in treponematoses.

[3] Philip. Journ. Sci. 35 (1928) pls. 18, 19, 20, 22, 23, and 24.
[4] Philip. Jour. Sci. 40 (1929) 91; 43 (1930) 263, 583.
[5] Philip. Journ. Sci. 42 (1930) 241.
[6] Philip. Journ. Sci. 42 (1930) 219.

TABLE 1.—*Showing the immunologic effect of antitreponematous vaccine therapy administered to yaws monkeys treated with neosalvarsan in the early stage of initial local lesion.*

[+, positive lesion; —, no lesion; ?, no lesion up to treatment; 0, not done. The animals were kept under observation six months after the last test for immunity.]

Designation of monkey.	Inoculation with yaws.		Neosalvarsan treatment.		Syphilis vaccine.
	Date.	Result.	Date.	Grams.	Date.
K-25	[a]XI-27-29	+	I-21-30	0.03	II-13-30
K-26	XI-27-29	+	I-21-30	0.03	II-13-30
K-27	XI-27-29	?	I-21-30	0.03	0
K-28	XI-27-29	+	I-21-30	0.03	0
Control	0		0		0
Do	0		0		0

Designation of monkey.	Tests for immunity by inoculation with yaws.			
	Date.	Result.	Date.	Result.
K-25	III-27-30	—	V-20-30	—
K-26	III-27-30	—	V-20-30	—
K-27	III-27-30	+	0	
K-28	III-27-30	+	0	
Control	III-27-30	+	0	
Do	0		V-20-30	+

[a] These letters and figures indicate month, day, and year: thus XI-27-29 means November 27, 1929.

ILLUSTRATION

TEXT FIGURE

FIG. 1. Showing the serologic and immunologic effect of antitreponematous vaccine therapy given to yaws monkeys cured by neosalvarsan in the early stage of initial local yaws. The serologic reactions were kindly performed by Dr. Onofre Garcia, of the division of biology and serum laboratory, Bureau of Science, and were read jointly by him and the author.

THE USE OF TETANUS ANATOXIN IN THE PROTECTION OF HORSES AGAINST INFECTION BY CHLOSTRIDIUM TETANI [1]

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Tetanus is not an uncommon disease among horses and mules in the United States Army, particularly in tropical countries. While the prevalence is not great enough to be considered alarming, the possibility of losing animals from tetanus, especially during military expeditions or periods of emergency, is of sufficient importance to make the development of some method of prophylactic immunization desirable. Consequently, experiments have been performed to determine whether horses can be "vaccinated" against tetanus with preparations of detoxified tetanus toxin, or "anatoxin," prepared according to the method of Ramon.(1)

The work of Ramon with his "anatoxins" suggested that this product might be used in a practical manner for immunization of large animals against tetanus. He states that it was formerly believed that in order to produce a lasting immunity in animals it was necessary to use an unmodified toxin for inoculation, but in contrast to this claim he has demonstrated that the injection of a toxin, particularly diphtheria toxin so modified that it is innocuous not only produces immunity but brings about a rapid and abundant production of antitoxin. In referring to the discovery of diphtheria "anatoxin" Ramon states that originally a small quantity of formaldehyde was added to the diph-

[1] From the United States Army Medical Department Research Board, Bureau of Science, Manila, Philippine Islands.

theria toxin for the purpose of preventing bacterial contamination, but that after several months it was noticed that toxicity gradually decreased while other features of this toxin remained. This led him and his associates to augment the quantity of formaldehyde to three or four parts of formalin per thousand of toxin. He stated that treating thus a toxin $_{100}$ of 1 cubic centimeter of which originally killed a 300-gram guinea pig in four days, they obtained, after one month a product 10 cubic centimeters of which produced no symptoms whatever in a guinea pig. Further, he stated that 1 cubic centimeter of this product, injected into a guinea pig, produced sufficient immunity after eighteen days to prevent death after the injection of several times a fatal dose of toxin, and after one month the animal was immune to from fifty to one hundred fatal doses. If two injections of 1 cubic centimeter each were made at intervals of three weeks, the animals were able to resist one thousand or more fatal doses after the last injection. Thus, the toxin after losing its toxicity, still retained its antigenic properties. Ramon calls attention to the fact that this method applies equally as well to the detoxication of tetanus toxin, and reported that experimental animals, which were inoculated with this product, were unaffected by the subsequent injection of lethal amounts of tetanus toxin.

In naturally acquired infections the tetanus spores are usually dry and probably contain little or no toxin. The spores alone would probably fail to cause infection were it not for the additional presence of pyogenic bacteria, as for instance staphylococci which produce a leukocidin and by its action on the leukocytes prevents destruction of the spores which then vegetate, multiply, and elaborate toxin.

It has been found to be practically impossible to remove toxin from spores in broth culture except by heating at 68° C. for five minutes. This simple procedure destroys toxin without causing death of the spores. Francis(3) states that washing of tetanus spores by successive suspensions in large quantities of salt solutions does not rid them of their toxin, but that heating of the spores at 80° C. for one hour renders them free from toxin, without impairing their infectivity. He further states that when toxin-free spores are injected into guinea pigs, tetanus does not result, but if quinine or staphylococci be injected simultaneously with the spores, tetanus and death follow promptly and regularly in from three to six days. He quotes

Semple, who stated that "when tetanus spores are carefully prepared and free from any trace of toxin or contamination of any kind they may be injected into susceptible animals without producing tetanus. Its success depends upon the fact that the phagocytes can pick up and digest tetanus spores in the absence of any irritant or other material likely to distract them, but when virulent spores mixed with toxin, other bacteria or other spores, sterile powdered charcoal, sterile sand, quinine, lactic acid, or anything which keeps away phagocytes, or occupies their attention, are injected hypodermically into susceptible animals, such as guinea pigs or monkeys, tetanus is the result."

The fact that recovery from infection usually does not confer immunity and that animals may be repeatedly reinfected have necessitated the frequent use of tetanus antitoxin when indicated.

According to Hutyra and Marek,(2) the horse possesses the greatest susceptibility for the virulent living cultures and toxin, and is followed by the guinea pig, goat, sheep, mouse, and rabbit. These authors state that "one cubic centimeter of the filtrate of a highly virulent culture kills a horse 500 Kgms. in weight; 0.001 cubic centimeter kills a guinea pig of 300 grams while other species of animals require proportionately larger doses of toxin. If for 1 gram of body weight of the horse the lethal dose is 1, guinea pigs require 2, goats 4, mice 13, rabbits 2,000, chickens 200,000 doses per gram of weight (Knorr)."

EXPERIMENTAL

In preparing the anatoxin, all the essentials of Ramon's method were followed. The technic is simple and merely involves the adding of three parts of commercial formalin to one thousand parts of liquid tetanus toxin followed by incubation at 37.5° C. for thirty days.

It was our desire to ascertain whether tetanus anatoxin would develop a sufficiently strong, active immunity to protect against infection with spores together with dirt or other foreign material such as is usually found in cases of natural origin.

IMMUNIZATION OF GUINEA PIGS WITH LIQUID TETANUS ANATOXIN LOT A

Experiment 1.—Lot "A" anatoxin was prepared from a toxin 0.0004 cubic centimeter of which killed a 350-gram guinea pig within three days. Three parts of commercial formalin per thousand of toxin were added, and the product was incubated

at 37.5° C. for thirty days. This was then injected into three guinea pigs as shown in Table 1.

From Table 1 it will be seen that three guinea pigs remained normal for over one month following injections of anatoxin in amounts which, before treatment with formalin, represented 5,000, 10,000, and 15,000 minimal lethal doses of tetanus toxin. A high degree of immunity was shown by these animals when at the end of one month they were tested by the inoculation of washed tetanus spores combined with unsterilized garden soil and *Staphylococcus albus*. In order to simulate natural infection a splinter dipped into the infective material was inserted in the site of inoculation. The control guinea pig (No. 4) developed tetanus on the day after injection and was destroyed on the second day. The vaccinated guinea pigs remained normal during an observation period of one month, after which they were discarded.

TABLE 1.—*Immunization of guinea pigs with liquid tetanus anatoxin Lot "A."*

Guinea pig No.	Tetanus anatoxin Injected; November 23, 1928.		Observation from November 23 to December 28, 1928.	Inoculated with lethal amounts of washed spores, etc.	Observation period for one month.		
	Amount.	Minimal lethal doses, prior to detoxication.			Dec. 30.	Dec. 31.	Jan. 28, 1929.
	cc.						
1	2.0	5,000	Normal...	Dec. 29, 1928	Normal...	Normal.....	Normal.
2	4.0	10,000	...do.....	Dec. 29, 1928	.....do.....	.....do.....	Do.
3	6.0	15,000	... do.....	Dec. 29, 1928	.... do....	.....do.....	Do.
4	0.0	0	.....	Dec. 29, 1928	Tetanus..	Destroyed....	

IMMUNIZATION OF GUINEA PIGS WITH DRIED TETANUS ANATOXIN LOT C

Experiment 2.—Lot "C" anatoxin was prepared in a similar manner to Lot "A." Minimal lethal dose of the toxin, prior to treatment, was 0.0004 cubic centimeter.

It was desired to ascertain if precipitated, desiccated, and powdered anatoxin would immunize animals as well as the liquid anatoxin. Therefore, 400 cubic centimeters of Lot "C" were saturated with ammonium sulphate, after which the precipitate was collected in an evaporating dish and worked with a spatula until all the solution had been removed. To the resulting gum-

my precipitate was added 6 grams of lactose; the mass was molded into a small pellet, and placed in vacuo in a desiccator over sulphuric acid. Six days later the dried anatoxin was removed and ground to a fine powder, of which the weight was 7.668 grams. As the 400 cubic centimeters of liquid anatoxin contained 1,000,000 detoxified minimal lethal doses of toxin and as there was a loss of approximately 25 per cent in handling, the 7.668 grams of powder represented approximately 750,000 detoxified minimal lethal doses and, accordingly, 1 gram represented about 97,809 detoxicated minimal lethal doses.

A portion of the powder was dissolved in sterile distilled water, and different amounts were injected into guinea pigs as shown in Table 2.

TABLE 2.—*Guinea-pig test of dried tetanus anatoxin Lot "C."*

Guinea pig No.	Tetanus anatoxin injected, March 25, 1929.		Observation from March 25 to April 23, 1929.	Inoculated with lethal amounts of washed spores, etc.	Observation period.	
	Amount.	Minimal lethal doses prior to detoxication.			April 24.	April 25.
	c.					
1	0.082	6,068	Dead [a]			
2	0.125	12,225	Normal	April 23, 1929	Tetanus	Dead.
3	0.250	24,450	do	April 23, 1929	do	Do.
4	0.500	48,900	do	April 23, 1929	do	Do.
5	0.0	0		April 23, 1929	Dead	
6	0.0	0		April 23, 1929	do	

[a] Cause undetermined.

One month later the guinea pigs were inoculated with tetanus spores in a manner similar to that followed in experiment 1. There were approximately 100,000 spores per cubic centimeter. All of the animals died two days later.

Comment.—From this experiment it appears that desiccated anatoxin, as prepared in this manner, possesses no immunizing properties. The "vaccinated" guinea pigs lived but one day longer than the controls.

Experiment 3.—Immunization of guinea pigs with dried tetanus anatoxin (Lots A and C).

One hundred cubic centimeters of liquid anatoxin "A" was saturated with ammonium sulphate and handled exactly as in the case of Lot "C" except that lactose was not added prior to desiccation.

Lot "A" possessed a minimal lethal dose of 0.0004 cubic centimeter and 100 cubic centimeters yielded 0.530 gram of powder; allowing for about 25 per cent loss in preparation, the 0.530 gram of powder should have contained approximately 187,500 detoxicated minimal lethal doses.

Desiccated Lot "A," without the addition of lactose, and desiccated Lot "C," which had been combined with lactose, were tested for immunizing properties by the injection of six guinea pigs. The results are set forth in Table 3.

One month after the injections with anatoxin these guinea pigs were inoculated with tetanus spores in a similar manner to the other lots, except that the spores were heated to 68° C. for five minutes to destroy any toxin present.

It is apparent from these results that the anatoxin was either lost during the precipitation and drying or was so modified that it was no longer capable of immunizing the animals tested.

IMMUNIZATION OF GUINEA PIGS WITH LIQUID TETANUS ANATOXIN LOT D

Experiment 4.—This lot of anatoxin was prepared from a toxin the minimal lethal dose of which was 0.0005 cubic centimeter. It was tested in the same manner as Lot "A."

It will be seen from Table 4 that while Lot "D" possessed some immunizing power, a dose of 4 cubic centimeters was necessary to prevent infection.

IMMUNIZATION OF HORSES AND MULES WITH LIQUID TETANUS ANATOXIN LOT A

Experiment 5.—The experimental results proving that guinea pigs were protected from infection with tetanus by anatoxin led to a similar investigation of the practical value of this agent in the immunization of larger animals, particularly horses and mules.

Nine animals at Fort William McKinley were, therefore, selected and injected subcutaneously with different doses of Lot "A" anatoxin. Three animals, Nos. 1, 4, and 7, were tested for immunity after the lapse of one month; three, Nos. 2, 5, and 8, after six months; and Nos. 1, 5, 6, and 7 after one year. With three exceptions all animals received a single dose of anatoxin; animals 7, 8, and 9 were given an additional injection thirty days after the first. Table 5 shows the results of the protection given by anatoxin in each of the animals used.

TABLE 3.—*Guinea-pig test of dried tetanus anatoxin Lots "A" and "C."*

LOT "A" (WITHOUT LACTOSE).

Guinea-pig No.	Tetanus anatoxin injected May 2, 1929. Amount.	Tetanus anatoxin injected May 2, 1929. Minimal lethal dose prior to detoxification.	Observation from May 2 to June 3, 1929.	Inoculated with lethal amounts of heated spores, etc.	Observation period. June 5.	June 6.	June 7.	June 8.	June 9.
	g.								
1	0.050	17,700	Normal	June 4, 1929	Stiff leg	Tetanus	Tetanus	Tetanus	Dead.
2	0.100	35,400	do	June 4, 1929	do	do	do	Dead.	
3	0.200	70,800	Dead *						
LOT "C" (WITH LACTOSE).									
4	0.125	12,225	Normal	June 4, 1929	Stiff leg	Tetanus	Tetanus	Dead.	
5	0.250	24,450	do	June 4, 1929	do	do	do	do	
6	0.500	48,900	Dead *						
7	0.0	0		June 4, 1929	Tetanus	Dead.			
8	0.0	0		June 4, 1929	do	do			

* These two guinea pigs had markedly inflamed adrenals and other signs of toxæmia.

TABLE 4.—*Immunization of guinea pigs with liquid tetanus anatoxin Lot "D."*

Guinea pig No.	Tetanus anatoxin injected; June 17, 1929.		Observation from June 17 to July 19, 1929.	Inoculated with lethal amounts of washed spores, etc.	Observation period.				
	Amount.	Minimal lethal doses prior to detoxication.			July 20.	July 21.	July 22.	July 23.	July 28.
	cc.								
1	2	4,000	Normal	July 19, 1929	Stiff leg	Stiff leg	Stiff leg	Tetanus (destroyed)	
2	4	8,000	do	July 19, 1929	do	do	do	Normal	Discarded.
3	6	12,000	do	July 19, 1929	do	do	do	do	Do.
4	0	0		July 19, 1929	do	Tetanus	Dead		
5	0	0		(a)	do	Normal	Normal	Normal	Discarded.

[a] Garden soil only.

TABLE 5.—*Immunization of horses and mules with anatoxin Lot "A."*

Animal No.	Brand.	Tetanus anatoxin Lot "A."				Inoculated with spores, etc.	Results.a	
		Date.	Amount injected.	Date.	Amount injected.			
			cc.		cc.			
1	a211W			Feb. 4, 1929	10	Mar. 9, 1929 Mar. 19, 1930 Mar. 31, 1930		Normal.
2	462W			Feb. 4, 1929	10	Aug. 22, 1929	Tetanus	Dead.
3	b02V8			Feb. 4, 1929	10	(c)		
4	122W			Feb. 4, 1929	20	Mar. 9, 1929	Tetanus	Dead.
5	037W			Feb. 4, 1929	20	Aug. 22, 1929 Aug. 19, 1930 Aug. 31, 1930		Normal.
6	008W			Feb. 4, 1929	20	Mar. 19, 1930 Mar. 31, 1930		Do.
7	22V0	Jan. 14, 1929	15	Feb. 4, 1929	15	Mar. 9, 1929 Mar. 19, 1930 Mar. 31, 1930		Do.
8	b01V6	Jan. 14, 1929	15	Feb. 4, 1929	15	(c)		
9	19V8	Jan. 14, 1929	15	Feb. 4, 1929	15	Aug. 22, 1929	Tetanus	Dead.

a "W" indicates a mule; all others are horses. b Animals destroyed for other reasons prior to the time set for the immunity test. c Not tested.

Animals 3 (02V8) and 8 (01V5), Table 5, were unfortunately ordered destroyed for other reasons prior to the time set for the immunity test. However, as shown in Tables 6, 7, and 8, substitutes were obtained later.

The first group of animals was tested one month after the administration of anatoxin (Table 6), animals 1 (211W) and 7 (22V0) showed no ill effects at any time during the test, and although No. 4 (122W) died, death did not occur until seventeen days following infection, while the two control animals died in five days.

The method of infecting these animals was far more severe than would probably occur in nature. The spores, about 77,000,000 to the cubic centimeter, were not heated, but washed twice with physiological salt solution, and contained preformed toxin. Spores mixed with a fresh suspension of *Staphylococcus albus* and fresh earth were injected into a subcutaneous tract previously made with a trocar. This was followed by the insertion of a splinter, which had been dipped into the mixture.

The results obtained with animals of the second group, tested six months after having received anatoxin, are shown in Table 7.

Mule 5 (087W) developed slight symptoms of tetanus August 30, eight days subsequent to infection, but was improved the following day and normal September 10. No. 2 (462W) showed symptoms August 26, four days after infection, and was destroyed on the 29th, seven days after infection. Horse 8 (19V8) showed symptoms August 30, eight days subsequent to infection, and was destroyed August 31, nine days after infection.

Both controls showed symptoms several days before the vaccinated animals; symptoms were pronounced in the controls on the 28th and 29th and they were destroyed.

The only differences between this test and that of March 9 (Table 6) were the length of time between vaccination and infection, and the number of spores injected. In the first group the test was made thirty-three days after vaccination, and 77,000,000 spores per cubic centimeter were injected, while in the second group, the test was made six months after vaccination and 200,000,000 spores per cubic centimeter were inoculated.

The following test of the third group, which took place one year subsequent to vaccination (February, 1929), included four animals, three of which had been tested for immunity on previous dates. These animals (Table 8) included all that re-

TABLE 6.—*Immunization of horses and mules with tetanus anatoxin Lot "A."*

[Tested one month after the last date anatoxin was administered.]

No.*	Brand.	Tetanus anatoxin Lot "A."				Inoculated with washed spores, etc.	Results.	
		Date.	Amount injected.	Date.	Amount injected.			
			cc.		cc.			
1	211W			Feb. 4, 1929	10	Mar. 8, 1929	No symptoms	Normal.
4	122W			Feb. 4, 1929	20	Mar. 9, 1929	Mar. 20, tetanus	Mar. 26, dead.
7	22V0	Jan. 14, 1929	15	Feb. 4, 1929	15	Mar. 9, 1929	No symptoms	Normal.
Control	141W					Mar. 9, 1929	Mar. 11, tetanus	Mar. 14, dead.
Do	9V96					Mar. 9, 1929	do	Do.

* See numbers in Table 5.

TABLE 7.—*Immunization of horses and mules with tetanus anatoxin Lot "A."*

[Tested six months after the last date anatoxin was administered.]

No.[a]	Brand.	Tetanus anatoxin Lot "A."				Inoculated with washed spores, etc.	Results.	
		Date.	Amount injected.	Date.	Amount injected.			
			cc.		*cc.*			
2	463W			Feb. 4, 1929	10	Aug. 22, 1929	Aug. 26, tetanus	Aug. 29, destroyed.
5	087W			Feb. 4, 1929	20	Aug. 22, 1929	Aug. 30, slight symptoms Aug. 31, normal Sept. 10, normal	Normal.
8	19V8	Jan. 14, 1929	15	Feb. 4, 1929	15	Aug. 22, 1929	Aug. 30, tetanus	Aug. 31, destroyed.
Control	022W					Aug. 22, 1929	Aug. 23, stiff gait Aug. 24, tetanus Aug. 29, marked tetanus	Aug. 29, destroyed.
Do	8V91					Aug. 22, 1929	Aug. 24, stiff gait Aug. 26, tetanus Aug. 28, marked tetanus	Aug. 28, destroyed.

[a] See numbers on Table 8.

TABLE 8.—*Immunization of horses and mules with tetanus anatoxin Lot "A."*

[Tested one year after last date anatoxin was administered.]

No.	Brand.	Anatoxin Lot "A."				Inoculated with washed spores, etc.	
		Date.	Amount injected.	Date.	Amount injected.	Date.	Result.
			cc.		cc.		
1	211W			Feb. 4, 1929	10	Mar. 9, 1929	Normal.
5	087W			Feb. 4, 1929	20	Aug. 22, 1929	Do.
6	006W			Feb. 6, 1929	20		
7	22V0	Jan. 4, 1929	15	Feb. 4, 1929	15	Mar. 9, 1929	Normal.
Control	47V6						

No.	Inoculated with heated spores, etc.[a]	Observation.		Inoculated with washed spores, etc.	Observation.		
		March 29.	March 30.		April 1.	April 2.	April 20.
1	Mar. 19, 1930	Normal	Normal	Mar. 31, 1930	Normal	Normal	Normal.
5	Mar. 19, 1930	do	do	Mar. 31, 1930	do	do	Do.
6	Mar. 19, 1930	do	do	Mar. 31, 1930	do	do	Do.
7	Mar. 19, 1930	Symptoms	do	Mar. 31, 1930	do	do	Do.
Control	Mar. 19, 1930	do	Tetanus[b]	Mar. 31, 1930	Tetanus	Marked tetanus; destroyed	

[a] March 19, 1930, spore culture heated at 68° C. for six minutes.

[b] Stiff gait, head, neck, and tail extended; reaction of nictitans membrane when head is elevated.

mained from the nine vaccinated one year previously. Two were previously destroyed because of a respiratory affection and were never subjected to an immunity test; therefore, the comparison should be made with seven rather than nine animals.

Three of the seven animals considered, Nos. 2, 4, and 8, showed no evidence of protection when *C. tetani* was injected. One year later the four remaining animals were again tested with the following results.

Animal 1 (211W) was injected with tetanus spores March 9, 1929; March 19, 1930; and again March 31, 1930, and survived.

Animal 5 (087W) was inoculated with tetanus spores August 22, 1929; March 19, 1930; and March 31, 1930, and remained normal.

Animal 6 (006W) was inoculated with tetanus spores March 19, 1930, and March 31, 1930, and remained normal.

Animal 7 (22V0) was inoculated with tetanus spores March 9, 1929; March 19, 1930; and March 31, 1930; and while it showed some slight symptoms March 28 and 29, it was normal on the 30th and on the 31st when it was again inoculated with *C. tetani*. It remained normal.

Experiment 2.—A second experiment, embracing seven vaccinated animals and one control, gave surprisingly different results, as none of these animals developed an immunity to tetanus following the administration of anatoxin (Table 9).

TABLE 9.—*Test of immunization of horses with tetanus anatoxin Lot "D."*

[Tested five and one-half months after anatoxin was administered.]

No.	Brand.	Tetanus anatoxin Lot "D."		Inoculated with washed spores, etc.	Results.	
		Date.	Amount injected.			
			cc.			
1	1150J	Oct. 24, 1929	25	Apr. 8, 1930	Tetanus	Destroyed, Apr. 15.
2	2V28	Oct. 24, 1929	25	Apr. 8, 1930	do	Destroyed, Apr. 17.
3	2V38	Oct. 24, 1929	25	Apr. 8, 1930	do	Destroyed, Apr. 16.
4	0307	Oct. 24, 1929	30	Apr. 8, 1930	do	Destroyed, Apr. 17.
5	2V61	Oct. 24, 1929	30	Apr. 8, 1930	do	Destroyed, Apr. 18.
6	2V74	Oct. 24, 1929	30	Apr. 8, 1930	do	Destroyed, Apr. 16.
7	C703	Oct. 24, 1929	30	Apr. 8, 1930	do	Destroyed, Apr. 17.
Control	0B15			Apr. 8, 1930	do	Destroyed, Apr. 16.

These animals had been injected with anatoxin Lot "D" approximately six months previous to being tested for immunity (Table 9).

The same infective material was used in this test as was employed in the first series. The spores were not heated.

No explanation can be made for the apparent total lack of immunity in these animals which had been injected with anatoxin about five and one-half months previously.

CONCLUSIONS

1. Immunization of guinea pigs against tetanus infection was easily accomplished by the injection of anatoxin prepared according to the technic of Ramon.

2. Precipitating, drying, and pulverizing of anatoxin resulted in a complete loss of the immunizing properties.

3. Tetanus anatoxin is harmless in large doses when given to large or small animals.

4. The results of experimental investigation of the protection afforded horses and mules by the injection of anatoxin were conflicting. In the first series, two of three animals were protected for a period of at least one month, one of three animals was protected for a period of about six months, while all of four animals tested one year after the administration of anatoxin were protected and one developed slight symptoms of infection but recovered. In the second series, seven horses died following the injection of tetanus spores, five and one-half months after the administration of anatoxin.

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EXPERIMENTAL STUDIES OF THE TREATMENT OF SURRA[1]

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NINE TEXT FIGURES

Surra, a term of Hindustani origin, meaning "rotten" or "spoiled," has been employed for many years to indicate a disease of certain wild and domesticated animals caused by *Trypanosoma evansi*.

The disease is characterized principally by fever; accelerated respiration; subcutaneous œdematous plaques and œdematous swellings of the genitals, limbs, and abdomen; marked emaciation despite an apparently good appetite; incoördination of gait; posterior paralysis and death.

The incubation period is from about four to fourteen days in natural infection and about five to eight days in cases that are deliberately induced.

Diagnosis is made by observation of clinical symptoms, by microscopic examination of peripheral blood for trypanosomes, and by the complement-fixation test.

Concerning the question of the actual cause of death of rats suffering with trypanosomiasis, Kligler, Geiger, and Comaroff (1) state that Schilling and Rondoni, Martin and Darre, Reichenow and Regendanz, and Tropp assume that the injury and ultimate death are due to a toxic substance liberated by the disintegration of the parasites. Schern and Fenyvessy hold that death is due to depletion of the blood sugar and glycogen reserve. Kligler

[1] From the United States Army Medical Department Research Board, Bureau of Science, Manila, Philippine Islands.

et al. state that while there is a general agreement that the blood-sugar concentration is decreased, no attention has been given to the possible harmful effects of intermediate products of sugar metabolism, and they are of the opinion that the lowered sugar concentration in the blood is due to its active utilization by the trypanosomes, and that this active glucose metabolism leads to a state of constant high lactic acid concentration in the blood. In experiments with normal and infected rats, they show that the increase in lactic acid is parallel to the rise in the number of trypanosomes, in some instances relatively enormous. This observation was carried out by tests with two sets of rats infected with *T. evansi*. One set was untreated, while twice daily the other received 0.5 cubic centimeter of a 10 per cent solution of bicarbonate of soda, intraperitoneally. The rats treated with soda bicarbonate lived half as long again as the untreated.

Edwards,(2) reporting his results in the treatment of surra with tartar emetic, states that the drug, when administered intravenously to a horse showing trypanosomes in its peripheral circulation, brings about disastrous results, the animal sometimes dying even before the termination of the injection. As examination of the peripheral circulation showed that the trypanosomes had disappeared, it was presumed that death had been caused by the occlusion of the capillaries with dead trypanosomes or, possibly, by the liberation of toxic products during their disintegration. He, therefore, recommended that the infected blood be initially freed of organisms either by administration of small amounts of tartar emetic or some other less drastic trypanocide, before more active treatment is begun.

The writers also have met with embarrassing results by the use of tartar emetic, but believe that such results were due to toxicity of the drug rather than to the occlusion of capillaries or to the liberation of toxic products from disintegrating trypanosomes. The deaths that occurred in certain of our cases followed in from one to three days after injections of the drug and at a time when trypanosomes had not been found in the peripheral circulation for a period of one month. The suggestion of Edwards, that certain animals appear to manifest an idiosyncrasy towards tartar emetic, is agreed with; but the writers, in harmony with Kligler and others, do not feel that death results from the liberation of toxic products, as the inoculation of the rats with large numbers of dead trypanosomes

as well as the rapid cure (five hours) of moribund rats teeming with trypanosomes, have failed to cause any symptoms of intoxication.

While it has been definitely proved that *T. evansi* may be carried in a mechanical manner by certain biting flies, there is no evidence to show that any cyclic development of the organism takes place within the bodies of such insects. According to Mitzmain,(3) the principal vector in the Philippines is *Tabanus striatus*. This observation was confirmed by Kelser,(4) who also incriminated the mosquito *Aëdes ægypti* as a mechanical carrier. Neither of these authors was successful in his endeavors to transmit the disease through the agency of *Stomoxys calcitrans*.

Surra occurs in India, Africa, Burma, Indo-China, Persia, Mauri, Java, Sumatra, and the Philippine Islands, and, owing to its fatal termination in horses, cattle, camels, goats, sheep, and dogs, it is of great importance to any country where such animals are factors in economic development. In the Philippines mules, horses, and native ponies are most susceptible, while the native carabao is relatively immune, and acts as a reservoir of the trypanosomes. However, the carabao may also succumb to the disease, if, for any reason, its vitality is lowered. Kelser,(4) in a survey in the Philippine Islands, embracing several hundred animals, found that over 50 per cent of the carabaos and more than 33 per cent of the native cattle showed evidence of harboring *T. evansi*.

Surra is rather widespread in the Philippines and in some localities is so prevalent as to prevent the maintenance of horses, so necessary to herding and other activities in connection with the cattle industry. During a period of twelve years 2,670 cases of surra were recorded in horses alone, to say nothing of the many cases not reported.

The United States Army in the Philippine Islands lost six horses and forty mules during an outbreak at Fort William McKinley in 1926; two mules at the same post in 1929; and twenty-six horses and two hundred eighty-nine mules at Fort Stotsenburg during an outbreak in 1929. At these posts the number of cases was kept at a minimum by isolation and strict quarantine of all animal suspects and by destruction and disposal of those known to be diseased.

Because of the ever present reservoirs of trypanosomes afforded by native cattle and the ubiquitous carabao, and the prev-

alence of insect vectors, surra in the Philippine Islands is a disease of serious importance to both civil and military activities.

For a considerable period of time before 1880, when Griffith Evans first discovered and proved the etiological significance of the flagellated protozoan in the blood of horses, mules, and camels suffering with surra, investigators had employed many drugs, alone and in combination, in an effort to discover an effective therapeutic agent for this disease.

Hornby,(5) commenting on the treatment of trypanosomiasis of cattle (Nagana), summarized his results as follows:

> Ten bulls were put into thick fly near Shinyanga Tanganyika Territory, and six were dead within three months and none survived eight months. Of ten bulls put into the same fly belt, but injected every fortnight of the first five months with 1 gram of tartar emetic, only two died within seven months; the remaining eight were in marketable condition, although infected with trypanosomes at the end of that period. Of ten bulls put in the same fly belt, but injected every fortnight of the first five months with a mixture of 2.5 gms. of Bayer 205 and 1 gram of tartar emetic, three were dead within seven months. Of the survivors one was very ill at the end of that time, but, the remaining six were in marketable condition though infected.

He concluded that "no advantage is obtained when Bayer 205 is added to the tartar emetic." Edwards,(2) in an article dealing with various investigations including his own, states that "according to Dale (1923), Cushny made the first suggestion of a trial of compounds of antimony and bismuth on account of their relationship with arsenic. Low (1916) in a review of the history of the use of tartar emetic in tropical medicine, stated that Nicholle and Mesnil (1906) first proposed the use of antimony salts in the treatment of trypanosomiasis." In his summary and conclusions, Edwards speaks highly of Bayer 205 as a trypanocide and calls attention to its ability to remain in the circulation for about two months, thus protecting the patient from relapses. He states, however, that the lack of diffusibility of the drug is a detracting feature as it does not reach the trypanosomes in the subarachnoid space. He overcame this shortcoming by administering dilute solutions of Bayer 205 intrathecally simultaneously with intravenous injections. He is of the opinion that Bayer 205 can be used alone with success, and that its action is superior to tartar

emetic. Edwards stated that results with tryparsamide were disappointing.

Tubangui(6) reported having successfully treated two native ponies with alternating doses of mercuric iodide and tartar emetic given intravenously, and with anthelmintics, the use of which was suggested by the theory that blood-sucking internal parasites may act as reservoirs and reinfect after termination of treatment.

Ch. Kahan Singh(7) reported encouraging results obtained with Bayer 205 and tartar emetic used in the treatment of surra in native ponies. He stated that of four animals treated at Sohawa, one died during medication while three were discharged as cured and were in good health at the time of his report. Thirteen out of fourteen cases admitted at Bhera received treatment and were alive and improving generally. Of forty-four cases admitted to the Veterinary Hospital at Quilla Sheikhupura, thirty-six received the full course of treatment, six died during medication, and two were taken away before completion of the therapy. Eight of the thirty-six discharged as cured relapsed, probably due to reinfection. The other twenty-eight were apparently normal. He also called attention to other observations which indicate that his method of treatment produced favorable results. All injections were made intravenously and were intended for the average-sized pony. The amount of drugs used, as well as the time intervals between injections, were as follows:

First day, 100 cubic centimeters of 2 per cent solution Bayer 205.
Sixth day, 100 cubic centimeters of 1 per cent solution tartar emetic.
Eleventh day, 150 cubic centimeters of 1 per cent solution tartar emetic.
Sixteenth day, 100 cubic centimeters of 2 per cent solution Bayer 205.
Twenty-first day, 150 cubic centimeters of 1 per cent solution tartar emetic.
Thirty-first day, 100 cubic centimeters of 2 per cent solution Bayer 205.

Stratman-Thomas and Loevenhart,(8) after commenting on the therapeutic value of a number of compounds that have been used as trypanocides, present data on the general biological actions of two of the six most promising drugs prepared by them. These two preparations were called etharsanol and proarsanol.

Etharsanol (drug "73"), or "para-oxyethylphenylarsenic acid," was first prepared by C. S. Hamilton in the laboratories of

Doctor Loevenhart. The arsenic content is 20.32 per cent; and it is stated that a 10 per cent solution of etharsanol can be heated in the sterilizer at 15 pounds pressure for thirty minutes without materially increasing its toxicity.

Stratman-Thomas and Loevenhart(8) state that ten to fourteen days after inoculation with trypanosomes the brain of untreated rabbits contained living organisms, as an emulsion of this brain tissue injected into rats produced trypanosomiasis. At the same time the blood was free of trypanosomes, as none could be found on careful microscopic examination, and the inoculation of the blood into rats failed to produce infection. However, it was also stated that following the injection of etharsanol the brain substance contained large amounts of arsenic and from this it was concluded that etharsanol is able to penetrate the tissues of the central nervous system and kill trypanosomes located there. These workers, who repeated the work of Voegtlin(9) and obtained similar results as with tryparsamide, state that etharsanol is equally as effective in clearing the cerebrospinal fluid of trypanosomes, when the technic described by Voegtlin, Smith, Dyer, and Thompson was used. Stratman-Thomas and Loevenhart (p. 476) concluded:

> The real test of a drug, however, is in animals where the infection is in the tissues, and not confined to the blood stream, and where therapeusis is started late in the disease. In rabbits, there seems to be little preference between the efficacy of etharsanol and tryparsamide. Etharsanol is capable of curing trypanosomal infections in rats and rabbits late in the disease and in some cases when the animal is near the point of death.

Other factors of importance are the relatively low cost of production of etharsanol and the excellent keeping qualities of this drug.

Two lots of etharsanol (Nos. 15 and 16) were furnished Vedder and Kelser(10) while they were members of the Medical Department Research Board, by Stratman-Thomas and Loevenhart, and are now being employed by the writers. This material is about four years old, and has been stored in loosely stoppered amber bottles and kept on a shelf in the laboratories of the Bureau of Science, Manila, Philippine Islands.

From the unpublished results of an experimental investigation of the treatment of surra by Vedder and Kelser,(10) it may be concluded that etharsanol (drug "73") is effective in the treat-

ment of rats infected with *T. evansi*, but that horses and mules failed to tolerate a curative dose and died of arsenical poisoning.

EXPERIMENTAL

The authors repeated the observations of Vedder and Kelser concerning the cure of infected rats by etharsanol and stimulated by its obvious trypanocidal action, continued the study of this drug in an endeavor to develop a method of administration by which its toxic action could be minimized and the disease cured in horses. In the present investigation etharsanol was used for the treatment of *T. evansi* infections in rats, horses, and mules.

DISTRIBUTION OF TRYPANOSOMA EVANSI IN THE TISSUES OF INFECTED RATS

In order to determine the location of trypanosomes in the various tissues of infected rats, which have received subtherapeutic doses of etharsanol resulting in apparent sterilization of the peripheral blood, the following experiments were carried out.

Experiment 1.—May 22, 1928. Four normal white rats were inoculated with *T. evansi*, and when trypanosomes appeared in the peripheral blood three days later three of the animals were injected intraperitoneally with subtherapeutic doses (20 milligrams) of etharsanol. On the following day (May 26) blood specimens from the three treated rats were free of organisms while the control remained positive. Rat 1 was killed May 26 and portions of heart, lymph glands, spleen, kidney, adrenal, liver, and bone were each macerated separately with physiologic salt solution and injected with separate syringes intraperitoneally into normal rats, as shown in Table 1–A. The results in Table 1 show that while the peripheral blood of rat 1 was free of trypanosomes, these organisms were present in all organs with the exception of the adrenals. The susceptibility of the rat injected with adrenal was proved by infection fifteen days later with *T. evansi*. The rat that received the extract of heart muscle died the following day from a puncture of the intestines.

Rat 2 had trypanosomes in the peripheral blood on the fifth day after the injection of etharsanol and died of the infection three days later, indicating that the 20-milligram dose was insufficient to effect a cure. Rat 3 showed similar results after seven days and died the following day. The control rat (No. 4), which was not treated, had trypanosomes in the blood from the third day after inoculation until death on the tenth day.

TABLE 1.—*Experiment 1: Result of inoculation of trypanosomes into four rats.*

[+, all infected May 22, 1928. Trypanosomes in blood.]

Rat No.	Trypanosomes in blood.	Injected with etharsanol May 26, 1928.	Blood examination.								
			May.						June.		
			26	27	28	29	30	31	1	2	3
		mg.									
1	May 25	20	—[a]								
2	May 25	20	—	—	—	—	+	+	+	Died.	
3	May 25	20	—	—	—	—	—	—	+	Do.	
4	May 25	(b)	+	+	+	+	+	+	+	Do.	

[a] Killed June 26. See Table 1–A. [b] Control.

TABLE 1–A.—*Normal rats inoculated May 26 with tissue extracts from rat 1 of Table 1.*

[D, died.]

Rat No.	Tissue.	Results.																			
		May.		June.																	
		30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	Heart	D[a]																					
2	Lymph gland	—	—	—	—	+	+	+	D														
3	Spleen	-		—	—	+	+	+	D														
4	Kidney	—	—	—	—	+	+	+	+	D													
5	Adrenal	-		—	—	—	—	—	—	—	—	—	—	—	—	—[b]	—	—	+	+	+	+	D
6	Bone	—	—	—	+	+	D																
7	Liver	—	—	—	—	—	—	—	+	D													
8	Tail blood	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[a] Punctured intestines. [b] Inoculated with *T. evansi*. [c] Discarded.

Experiment 2.—August 31, 1928. Three rats were infected with *T. evansi* and all had trypanosomes in their blood September 4, at which time rats 1 and 2 were each given 20 milligrams of etharsanol intraperitoneally.

The control rat, No. 3, which had not been treated, had trypanosomes in the blood from the fourth day after infection until death on the eighth day.

The blood of rat 2 showed no trypanosomes on the first, second, third, fourth, or fifth day after treatment, but was positive from the sixth to the tenth day when it died of the infection.

Rat 1 had no demonstrable trypanosomes in the blood during the two days following treatment. This animal was killed on

TABLE 2.—*Experiment 2: Result of inoculation of trypanosomes into three rats.*

[D, died. All rats infected August 31, 1928.]

Rat No.	Trypanosomes in blood.	Injected with ethar-anol Sept. 4, 1928.	Results. September.									
			4	5	6	7	8	9	10	11	12	13
		mg.										
1	Sept. 4	20	+	—	[a]							
2	Sept. 4	20	+	—	—	—	—	—	+	+	D	
3	Sept. 4	(b)	+	+	+	+	D					

[a] Killed September 6 for rats in Table 2-A. [b] Control.

TABLE 2-A.—*Normal rats inoculated September 6 with tissue extracts from rat 1 of Table 2.*

[D, died.]

Rat No.	Tissue.	Results. September.												
		9	10	11	12	13	14	15	16	17	18	19	20	21
1	Heart	—	—	—	—	—	—	—	+	+	D			
2	Lymph glands	—	—	—	—	—	—	—	—	—	—	—	—	—
3	Spleen	—	—	—	—	—	—	—	—	—	—	—	—	—
4	Kidney	—	—	—	—	—	—	+	+	+	+	+	+	+
5	Adrenal	—	—	—	—	+	+	+	+	D				
6	Bone	—	—	—	—	—	—	—	—	—	—	—	—	—
7	Liver	—	—	—	—	—	+	+	D					
8	Brain	—	—	—	—	—	—	—	—	—	—	+	+	+
9	Tail blood	—	—	—	—	—	—	—	—	—	—	—	—	—

Rat No.	Tissue.	Results. September.								October.				
		22	23	24	25	26	27	28	29	30	1	2	3	5
1	Heart													
2	Lymph glands	—	—	—	—	—	—	—	—	—	+	+	+	D
3	Spleen	—	—	+	+	+	D							
4	Kidney	+	D											
5	Adrenal													
6	Bone	+	+	+	D									
7	Liver													
8	Brain	D												
9	Tail blood	—	—	—		—	—		—	—	+	+	+	D

[a] Inoculated with *T. evansi*.

the latter day and the various organs were removed, suspended in salt solution, and inoculated into normal rats. As shown in Table 2–A trypanosomes were still present in the heart, spleen, kidney, adrenal, bone, liver, and brain.

From these results it may be seen that while a subtherapeutic dose of etharsanol temporarily freed the peripheral circulation of trypanosomes, the organisms were still present in various internal organs.

Experiment 3. To test the effectiveness of etharsanol and tartar emetic used in subtherapeutic doses for treatment of rats infected with T. evansi.

The work of Vedder and Kelser demonstrated that the peripheral blood of horses given a subtherapeutic dose of etharsanol remained free of detectable trypanosomes for a period of only about eleven days. It seems probable that during this period the organisms must be in the internal organs. From the reports of Stratman-Thomas and Loevenhart, it appears that etharsanol has the property of invading the meninges and spinal fluid, while the reports of many investigators indicate that tartar emetic exerts a drastic effect upon trypanosomes in the peripheral circulation. Thus it was considered probable that if blood could be sterilized with tartar emetic and if the trypanosomes located in the spinal canal, deeper tissues, and organs could be reached by etharsanol, and the administration of the drugs so regulated as to prevent a cumulative action, the disease might be cured. Therefore, in this experiment white rats infected with *T. evansi* were treated with relatively small doses of tartar emetic alternating with etharsanol at intervals of about five days. The largest dose of etharsanol given was 20 milligrams, which is approximately one-third the amount required as a single dose to cure infected rats.

June 15, 1929. Ten normal white rats were inoculated with *T. evansi*. Four days later trypanosomes were present in the blood of all these animals. On this day eight of the rats were treated with different amounts of etharsanol, one was treated with tartar emetic, and one untreated animal was kept as a control. The control animal died on the seventh day after inoculation. Rat 9, which had been given 1 cubic centimeter of a 1 per cent solution of tartar emetic, died in twenty-four hours, apparently due to the toxicity of the drug, which prompted the use of a weaker solution for subsequent injections. Rat 8, treated with one dose of etharsanol (20 milligrams), showed

no trypanosomes in the blood for four days and then became positive and remained so until the tenth day when death occurred. Rat 1, which was given 5 milligrams of etharsanol, died the following day from an undetermined cause. The six remaining rats, Nos. 2, 3, 4, 5, 6, and 7, received alternating doses of etharsanol and tartar emetic, as shown in Table 3.

No trypanosomes were found in the blood of rat 2 during the period from June 30 to August 22, or fifty-three days; rat 5 showed no trypanosomes during the period from June 30 to August 1, or forty-three days; and the same was true of rats 3, 4, and 6 from July 6 to August 22, or forty-seven days. August 22 all live rats were inoculated with *T. evansi* to determine their susceptibility and all died from surra.

From the above results it would appear that while 20 milligrams of etharsanol alone were not sufficient, 15 milligrams of etharsanol and 1 cubic centimeter of a 1 to 300 solution of tartar emetic, in alternate doses were enough to cure *T. evansi* infection in the average white rat weighing from 180 to 200 grams.

The observation that small doses of etharsanol, in combination with tartar emetic, were sufficient to produce a permanent cure in rats indicated that perhaps a similar procedure might prove effective in the treatment of larger animals. Consequently, eight horses and one mule, all condemned animals, were obtained from the Quartermaster, Philippine Department, and used for experimentation. They were kept at the laboratory of the veterinary research division, Philippine Bureau of Animal Industry.

It might be well to state that in treating animals with tartar emetic, care should be exercised that none of the drug escapes into the subcutaneous tissues. Should this occur, extensive, painful swellings result, making it difficult to locate the jugular at the time of subsequent treatments, to say nothing of the apparent discomfort to the animal.

It has been our practice to dissolve the drug in distilled water immediately prior to intravenous injection and administer the solution slowly, and intermittently. Upon completion of the injection, the jugular vein was dammed and a quantity of blood was allowed to flow out through the needle, thus washing it clean of the drug before withdrawal.

Temperatures of nine normal horses, taken at 6 a. m. for a period of one week during February showed an average of 100.2° F. The highest was 100.4° F. and the lowest 98.1° F.

The majority showed temperatures of 100° F. or more. Temperatures of ten normal animals, taken in the evening for a period of one week during the month of February, showed an average temperature of 99.7° F. The highest was 101° F. and the lowest 99° F. In twenty-seven instances the temperature was 100° F. or more, while the remaining forty-three temperatures were above 99° F.

A modification of Broden's(11) method was employed to detect small numbers of trypanosomes microscopically. From 5 to 10 cubic centimeters of blood was allowed to flow from the jugular vein into several cubic centimeters of physiological salt solution containing 1 per cent sodium citrate. This was centrifuged at high speed, thus forcing the erythrocytes and trypanosomes to the bottom. The trypanosomes being of lower specific gravity than the cells, were found in the thin surface of the precipitate. A representative portion of the upper stratum was removed with a pipette and examined in the moist state with the high dry lens. Trypanosomes are easily demonstrated by this method, and the chances for their detection are many times greater than by the single-drop method. Such examinations were made each day during the experiment.

The complement-fixation test for surra was employed in conjunction with the microscopic examination of peripheral blood. The antisheep hæmolytic system, using one and one-half units of amboceptor and two units of complement was used. The antigen, a fresh suspension of *T. evansi*, was prepared after the method of Reynolds and Schoening.(12) In recording the results of the complement-fixation tests in the tables, the following symbols were employed: 4 +, complete fixation of complement; 3 +, 75 per cent fixation; 2 +, 50 per cent fixation, 1 +, 25 per cent fixation; + −, less than 25 per cent fixation; −, complete hæmolysis; and a dotted line indicates no test that particular day.

When the experimental animals were killed specimens from either brain, spleen, or spinal fluid were mixed with salt solution, and 1 cubic centimeter amounts were injected into white rats. After the rats had remained free of trypanosomes for twenty days they were then inoculated with *T. evansi* in order to demonstrate their susceptibility.

Charts and protocols explaining the daily observations on each experimental animal follow in chronological order.

TABLE 8.—*Rats inoculated with Trypanosoma evansi June 15, 1939, and treated with etharsanol (drug "73") and tartar emetic.*

[0, no treatment; +, trypanosomes in peripheral circulation; —, no trypanosomes in peripheral circulation; D, dead; TE, tartar emetic; drug "73," etharsanol.]

No.	June.													July.						
	19		20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	
	Drug "73."						TE 1-500.					Drug "73."						TE 1-500.		
		g.					cc.					g.						cc.		
1	+	0.005	D																	
2	+	0.006	+		—	—	+	1.0	—	—	—	+	0.01	—	—	—	—	—	—	1.0	—
3	+	0.007	+	+	+	+	+	1.0	—	—	—	+	0.01	—	—	—	+	+	+	1.0	+
4	+	0.008	+	—	—	—	+	1.0	—	—	—	+	0.01	—	—	—	—	+	+	1.0	—
5	+	0.009	+	—	—	—	+	0.5	—	—	—	+	0.01	—	—	—	—	—	—	1.0	—
6	+	0.010	—	—	—	—	+	0.5	—	—	—	—	0.01	—	—	—	—	+	+	1.0	D
7	+	0.015	+	—	—	—	+	0.5	—	—	—	—	0.01	—	—	—	+				
8	+	0.020	+	—	—	—	—	0	+	+	+	+	D								
9	+	(a)	D																		
10	+	0	+	+	D																

TABLE 3.—*Rats inoculated with Trypanosoma evansi June 15, 1929, and treated with etharsanol (drug "73") and tartar emetic*—Continued.

[0, no treatment; +, trypanosomes in peripheral circulation; —, no trypanosomes in peripheral circulation; D, dead; TE, tartar emetic; drug "73," etharsanol.]

No.	July.								August.											
	7	8	9 Drug "73."	10	11	12	13 TE 1-300.	July 13 to Aug. 1.	1	2 to 22	22	23	24	25	26	27	28	29	30	31
			g.				cc.													
1																				
2	—	—	—	0.015	—	—	—	1.0	—	—	—	(b)	—	—	—	—	+	+	+	D
3	—	—	—	0.015	—	—	—	1.0	—	—	—	(b)	—	—	—	—	+	+	+	+	D
4	—	—	—	0.015	—	—	—	1.0	—	—	—	(b)	—	—	—	—	+	+	+	+	D
5	—	—	—	0.015	—	—	—	1.0	—	D										
6	—	—	—	0.015	—	—	—	1.0	—	—	—	(b)	—	—	—	—	+	+	+	+	D
7																				
8																				
9																				
10																				

[a] One cubic centimeter of 1 per cent tartar emetic.

[b] Infected with *T. evansi*.

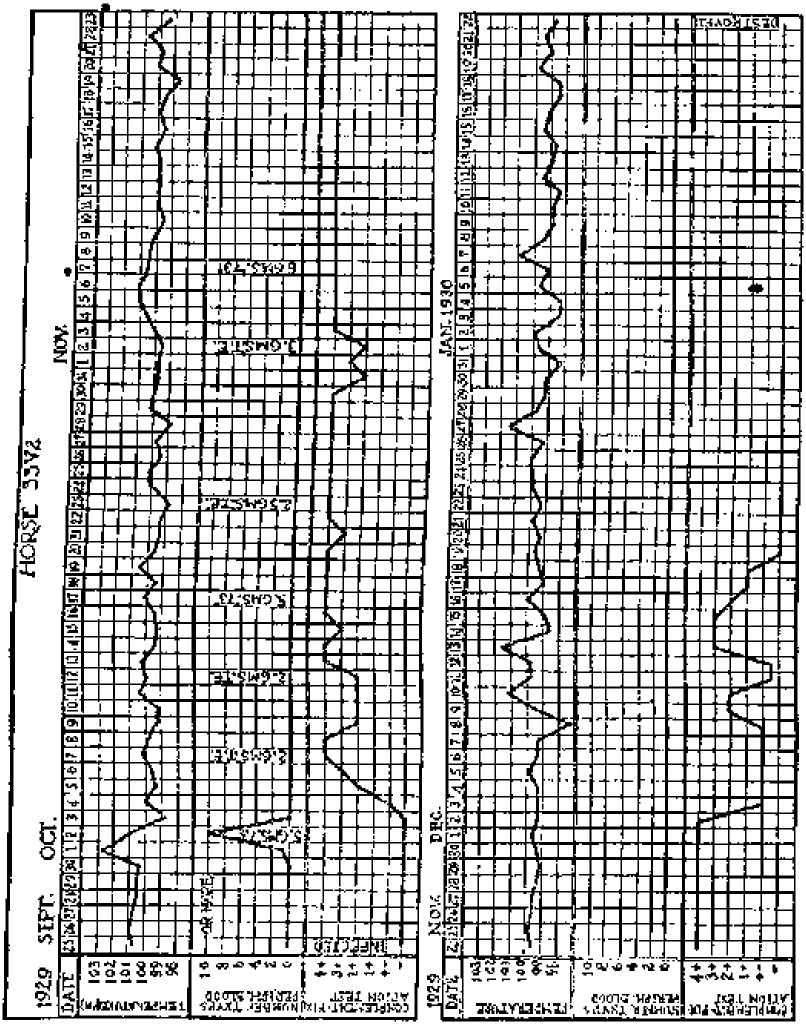

FIG. 1. Chart of horse 33V2.

EXPERIMENT 1

Horse 33V2. Inoculated with *T. evansi* September 25, 1929. Trypanosomes appeared in the peripheral circulation October 1, or six days subsequently. October 2 treatment was commenced with a 5-gram dose of drug "73." Treatment continued until November 7, when the last dose was given. Trypanosomes were not in evidence in the circulation the day following the initial treatment October 2, nor did they again appear during the experiment. Complement-fixing bodies appeared in the serum October 4 and continued in varying amounts until December 18, when the serum became negative and remained so to the termination of the experiment December 22, a period of thirty-five days. The daily temperature fluctuated considerably, but in the Tropics there is a wide variation in normal animals, and unless pyrexia is relatively high and associated with other clinical signs, such temperatures are of little significance.

This animal was apparently normal after the first treatment. No trypanosomes were found in the peripheral blood from the time of first treatment, a period of one hundred twelve days.

At the termination of the experiment January 22, 1930, blood, spinal fluid, brain, and spleen tissue were injected into white rats. They remained normal for twenty days, when their susceptibility was proved by inoculation with *T. evansi*.

Comment.—In view of the fact that the temperature remained normal after the first treatment, or one hundred twelve days; that trypanosomes were not found during a period of one hundred twelve days; that the complement-fixation test remained negative for thirty-five days; that the general health of the animal was excellent; that the blood, spinal fluid, brain, and spleen pulp were free from trypanosomes, it is believed that this case was cured by the treatment used.

EXPERIMENT 2

Horse 35V7. Weight 850 pounds. Inoculated with *T. evansi* November 18, 1929, and the peripheral blood showed trypanosomes November 25, or seven days subsequently. This animal was permitted to go untreated until December 4, or nine days after trypanosomes made their appearance. On the morning of, December 4, it was given 8 grams of drug "73" and the blood was free of trypanosomes by the afternoon of the same day, and remained negative thereafter. December 13, nine days later, this horse was given 3 grams of tartar emetic and died the

day and remained so for the duration of the experiment. Complement-fixing bodies appeared February 19, or eight days following infection and two days following the appearance of trypanosomes in the circulation, and with the exception of a short period between March 1 and 6, inclusive, were present in varying amounts. March 24, the dose of tartar emetic was increased to 3 grams. The animal remained normal in appearance until March 29, when it showed general choreic symptoms with profuse sweating, and died suddenly.

Trypanosomes were absent from the circulation the date of the first treatment until death, twenty-nine days later.

White rats injected with material from the brain, spinal fluid, and spleen pulp remained normal for twenty days, when their susceptibility was tested by inoculating with *T. evansi*.

Comment.—No trypanosomes were found in this animal from the beginning of treatment until death, which was probably due to poisoning by tartar emetic.

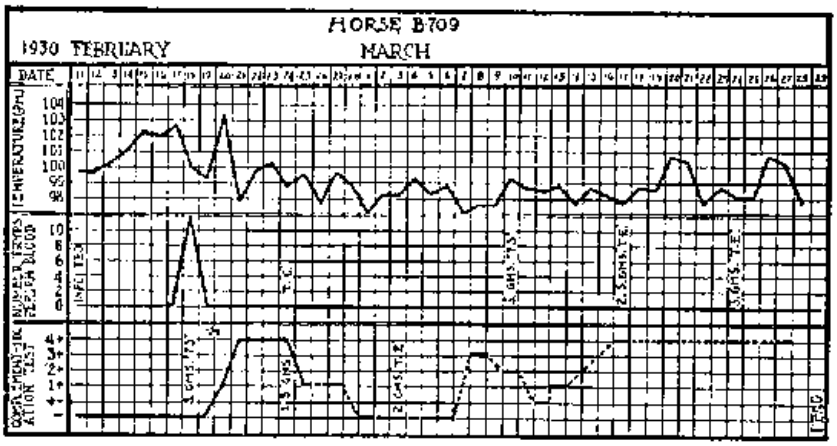

FIG. 4. Chart of horse B–709.

EXPERIMENT 5

Horse 32V6. Weight 900 pounds. Infected with *T. evansi* February 11, 1930. Trypanosomes appeared in the peripheral blood February 16, or six days after infection. Treatment was begun February 18, the second day after peripheral invasion. No trypanosomes were found the following day, nor at any time thereafter. March 24, 3 grams of tartar emetic were administered and the following day the animal showed marked cho-

reic symptoms of head and neck, with profuse sweating, and died within a few hours.

Trypanosomes were not found from the day following first treatment, a period of thirty-five days.

White rats injected with spleen pulp and spinal fluid collected at the time of death, remained normal for twenty days, when their susceptibility was tested by injection with *T. evansi*.

Comment.—No trypanosomes were found in this animal from the beginning of treatment until death, which was probably due to poisoning by tartar emetic.

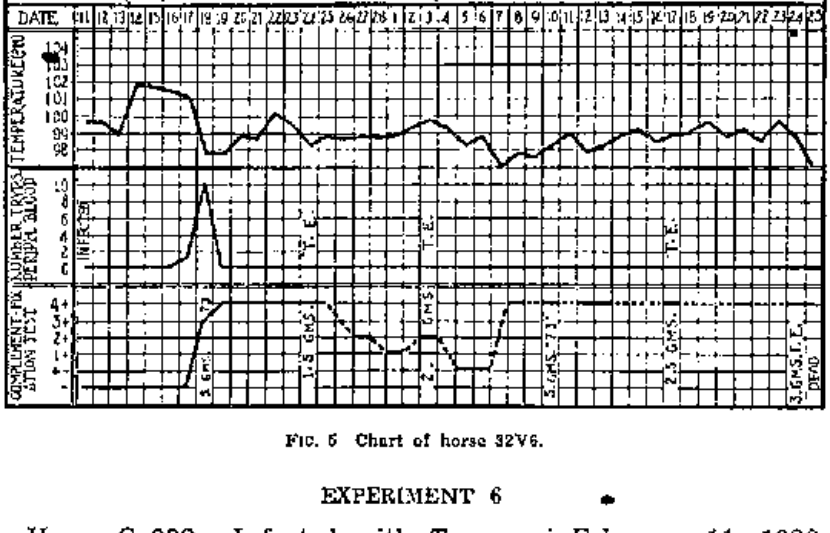

FIG. 6 Chart of horse 32V6.

EXPERIMENT 6

Horse C–932. Infected with *T. evansi* February 11, 1930. Trypanosomes appeared in the peripheral blood February 17, or the sixth day after infection. Treatment was commenced the day following, February 18. The trypanosomes disappeared and the circulation remained free thereafter, a period of thirty-six days. Complement-fixing bodies appeared February 18, seven days after infection and one day following the appearance of trypanosomes in the circulation. March 24, 3 grams of tartar emetic were administered, which proved too great a dose. Two days later, March 26, the animal was sweating profusely, and after showing some muscular twitching, died.

White rats inoculated with spleen pulp and spinal fluid from this animal, remained normal for twenty days when their susceptibility was tested by infection with *T. evansi*.

Comment.—No trypanosomes were found in this animal from the beginning of treatment until death, which was probably due to poisoning by tartar emetic.

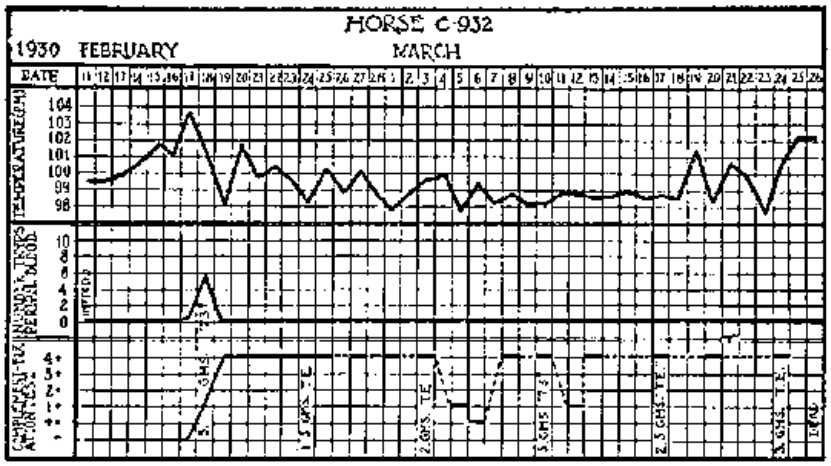

FIG. 6. Chart of horse C-932.

EXPERIMENT 7

Horse 06V8. Age 20 years. Weight 1,250 pounds. This animal was infected with *T. evansi* February 11, 1930. Trypanosomes appeared in the peripheral circulation February 17, or six days following infection. Treatment commenced on the following day, and the peripheral blood was negative for trypanosomes February 19 and continued thus until April 21, when this animal suffered from a relapse. Treatment was again given February 22. The blood was negative the following day and remained so up to the date of destruction of the animal, May 13. April 16, or five days prior to the reappearance of trypanosomes, the complement-fixation test, which had been plus-minus for six days, suddenly became four plus and continued at that level until termination of the experiment. White rats injected with spleen pulp and spinal fluid from this horse at the time of destruction, May 13, remained normal for twenty days when their susceptibility was proved by inoculation with *T. evansi.*

Comment.—It is apparent that in this particular case the initial treatment was not sufficient to destroy all the trypanosomes, as a relapse occurred sixty days after the last injection of the drugs. Had the course of chemotherapy been continued for a

few weeks longer, a cure might have been effected. The value and the accuracy of the complement-fixation test are demonstrated in this case.

EXPERIMENT 8

Horse 05V9. Age 20 years. Weight 1,500 pounds. Infected with *T. evansi* February 11, 1930. Trypanosomes appeared in the peripheral blood February 17, the sixth day after infection. Treatment was commenced February 18, one day following the appearance of trypanosomes. The day following treatment the peripheral circulation was negative for trypanosomes and remained so during the remainder of the experiment, or a period of eighty-three days.

Complement-fixing bodies first appeared February 18, the seventh day following infection and the day following appearance of trypanosomes in the peripheral blood. After fluctuating for forty-two days, the test became negative and remained so during the rest of the experiment, a period of forty-two days.

May 13 the animal was destroyed, and spinal fluid and spleen pulp were injected into white rats. The rats remained normal for a period of twenty days when their susceptibility was tested by the inoculation of *T. evansi*.

Comment.—In view of the fact that the temperature remained normal after the first treatment (eighty-one days); that trypanosomes were not found during a period of eighty-four days; that the complement-fixation test remained negative for forty-one days; that the general health of the animal was excellent; and that the spinal fluid and spleen pulp were free of trypanosomes, it is believed that this case was cured by the treatment used.

EXPERIMENT 9

Horse C936. Age 10 years. Weight 1,025 pounds. Infected with *T. evansi* February 11, 1930. Trypanosomes made their appearance February 17, or six days after infection. February 18 treatment was commenced. No trypanosomes were found in the peripheral circulation the day following first treatment, nor were any found during the remainder of the experiment, a period of eighty-four days.

Complement-fixing bodies were demonstrated in the serum the seventh day following infection and one day after trypanosomes were found in the circulation. After some fluctuation the fixation test became negative April 8 and remained so for the duration of the experiment, a period of thirty-five days.

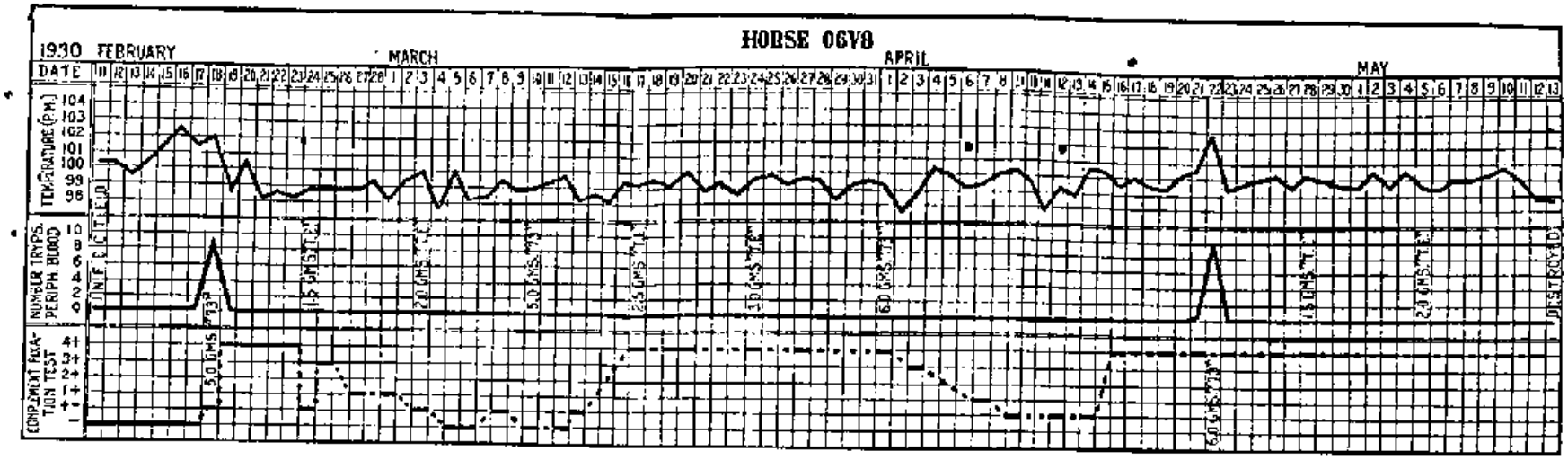

FIG. 7. Chart of horse 06V8.

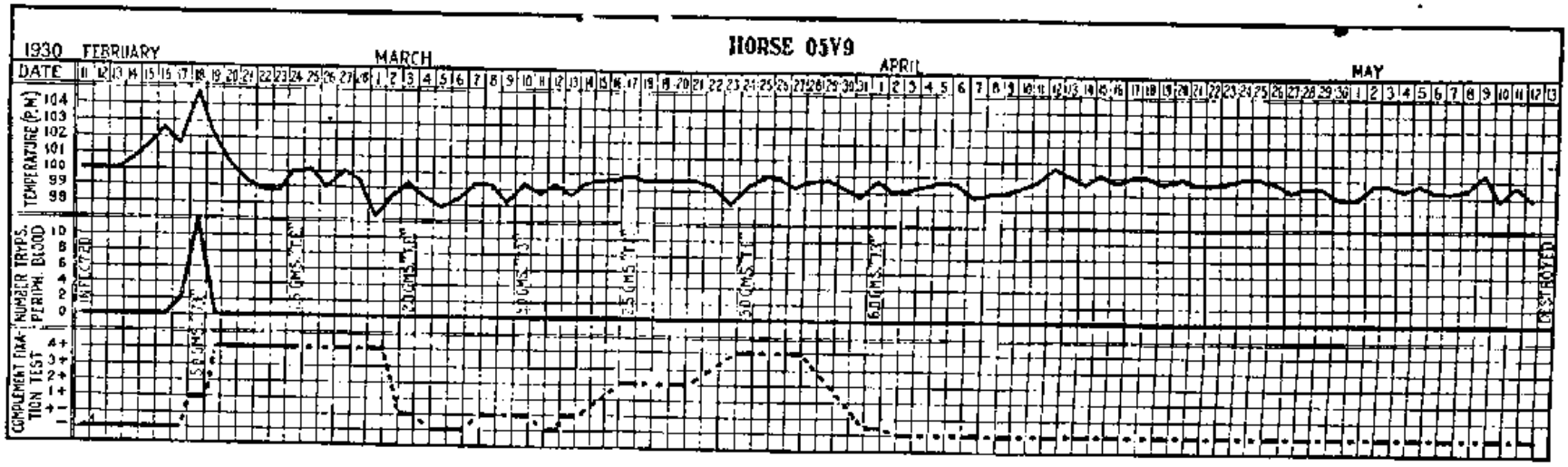

FIG. 8. Chart of horse 05V9.

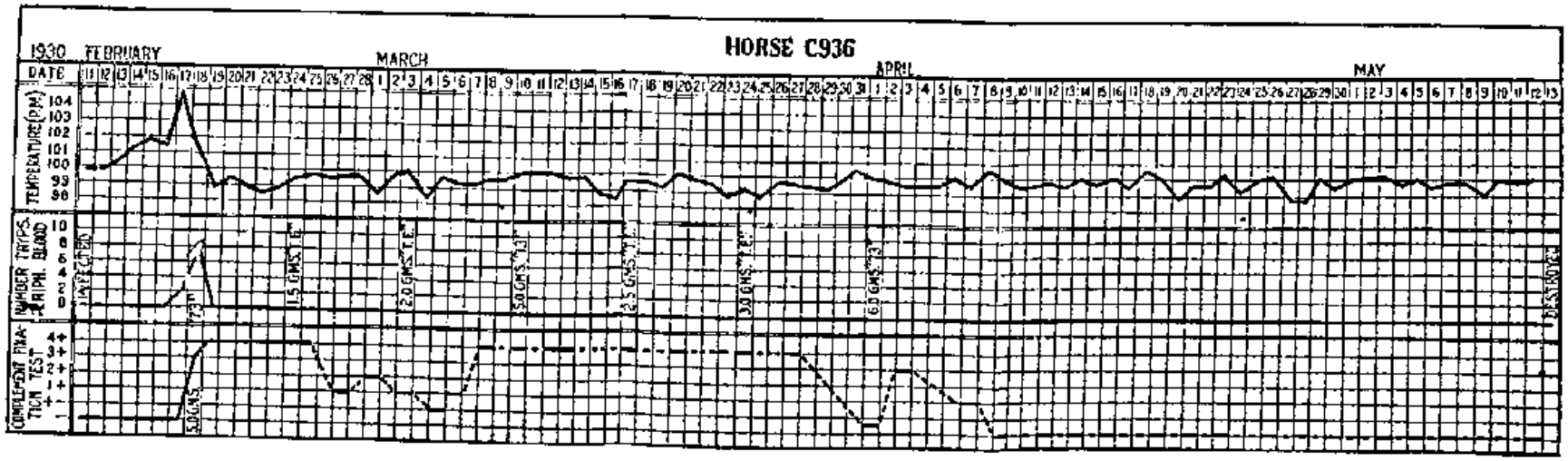

FIG. 9. Chart of horse C936.

The animal was destroyed May 13, and white rats were injected with spleen pulp and a part of the spinal fluid. All rats remained normal for twenty days, when their susceptibility was tested by injection of *T. evansi.*

Comment.—As the temperature remained normal for approximately three months following initial treatment; as no trypanosomes were found in the peripheral circulation following the first treatment; as the complement-fixation test remained negative for a period of thirty-five days; as no trypanosomes could be demonstrated in emulsion of the spleen and spinal fluid by animal inoculation; and as the general health of this animal was excellent at all times, it is concluded that the treatment given this animal effected a cure.

SUMMARY AND CONCLUSIONS

1. It was shown that rats infected with *T. evansi* may be cured by alternating injections of tartar emetic and etharsanol in doses smaller than the amount required when the latter drug is used alone.

2. The use of larger amounts of these drugs in the treatment of infected horses and mules gave results which are considered to be of sufficient promise to warrant further study.

3. Five animals, each of which weighed less than 1,000 pounds, died during treatment, probably due to poisoning by tartar emetic. However, none of these animals had trypanosomes in the blood at any time after the first treatment; and the organisms were not demonstrable in their spinal fluid, or in certain of their organs, extracts of which were inoculated into susceptible white rats.

4. In one experiment the blood remained free from trypanosomes for sixty days, after which a relapse occurred. However, following additional treatment the blood again became negative and remained so for twenty days, after which the animal was killed. Suspensions of the spinal fluid and spleen injected into susceptible rats failed to produce infection.

5. In three experiments the animals were apparently cured of surra, as indicated (*a*) by the immediate disappearance and permanent absence of trypanosomes from the blood, (*b*) by the fact that symptoms disappeared and the temperature remained normal, (*c*) by the findings that the complement-fixation test became negative and remained so, and (*d*) by the fact that suspensions of the spinal fluid and spleen injected into susceptible rats failed to cause infection.

6. In view of the fact that a relapse occurred in one animal after a period of sixty days, it seems important that further experimental studies be made to so modify the course of treatment either by prolonging it or preferably by increasing the amounts of etharsanol, that the possibility of relapses may be prevented.

ACKNOWLEDGMENTS

The writers acknowledge their indebtedness to Maj. Gen. Douglas MacArthur, commanding general, Philippine Department; Col. H. H. Rutherford, the surgeon, Philippine Department; Lieut.-Col. Charles H. Patterson and Maj. Roy Henry, of the Inspector General's Department; the Quartermaster Department, Manila and Fort William McKinley; Capt. George J. Rife, V. C., and Lieut. Verne C. Hill, V. C., for their interest and assistance; to Dr. William H. Brown, director of the Bureau of Science, Manila, for his help in furnishing material for screening stalls for maintenance of the experimental animals; to Dr. Eugene Rodier, chief of the veterinary research division, Philippine Bureau of Animal Industry, for his coöperation in furnishing stables and other facilities; and to Joseph M. Hoff, private 1st class (specialist 1st class), Medical Department, United States Army, for his assistance during the experimental work.

The authors are especially grateful to Dr. Estefano C. Farinas, of the veterinary research laboratory, Philippine Bureau of Animal Industry, for valuable help in carrying out the experiments.

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ILLUSTRATIONS

TEXT FIGURES

ANALYSIS AND FOOD VALUE OF SOME UNUSUAL PHILIPPINE FRUITS

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and

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NINE PLATES

Several papers on the composition of the common Philippine fruits [1] have been published. The object of the present investigation was to determine the composition of several unusual Philippine fruits that are not commonly known, and are used by Filipinos as food only in certain localities.

EXPERIMENTAL PROCEDURE

Analyses of these Philippine fruits were made according to the methods of the American Association of Official Agricultural Chemists and also Leach's Food Analysis.

When the fruits were received they were allowed to ripen, if they were not yet ripe, and were then weighed as whole fruits. In preparing samples for analysis the peelings and seeds were removed from the larger fruits and discarded and the edible portion weighed and ground to a uniform mass. In preparing samples of the very small fruits the whole fruit, including the seeds and peelings, were ground to a uniform mixture. As customary in food analysis the common constituents were determined individually, but the percentage of carbohydrates other than sugars and crude fiber was obtained by

[1] Santos, F. O., and F. T. Adriano, Bull. Philip. Public Welfare Commission, Manila (1928). Wester, P. J., Bull. Philip. Bur. Agr. 39 (1925). Pratt, D. S., and J. I. del Rosario, Philip. Journ. Sci. § A 8 (1913) 59. Wells, A. H., F. Agcaoili, H. Taguibao, and A. Valenzuela, Philip. Journ. Sci. 36 (1928) 157. Valenzuela, A., and P. J. Wester, Philip. Journ. Sci. 41 (1930) 85. Salvador W., Philip. Journ. Sci. 20 (1922) 363.

...ch... ...e total percentage of the other constituents from 100. Analyses of these fruits are given in Tables 1 and 2.

DESCRIPTION OF FRUITS

ANONA sp. Atemoya. Plate 1, fig. 1.[2]

A small deciduous tree of rapid growth adapted to low and medium altitudes, succeeds well where the dry season is pronounced.

The leaves and flowers are similar to those of cherimoya. The fruits are somewhat like the cherimoya in appearance but smaller, and are sweet, juicy, subacid, and of a good to excellent quality, but in most seedlings a shy bearer. A hybrid between the cherimoya and sugar apple. Fruited for the first time at Lamao experiment station in 1913.

ARTOCARPUS ELASTICA Reinw. Gomihan. Plate 1, fig. 2.

A tree of medium to large size, sometimes more than 15 meters high, growing at low altitudes in regions with abundant rainfall of equal distribution, from southern Luzon to Mindanao. The leaves are large, entire or trilobed, dark green, and rather rough beneath. The fruit is roundish, about 10 centimeters across, orange-yellow, and covered with long coarse threadlike hairs. The flesh is whitish, sweet, juicy, aromatic, and of good taste but rather scant. The seeds are roasted and eaten like peanuts.

ARTOCARPUS ODORATISSIMA Blanco. Maráng. Plate 2, fig. 1.

A handsome tree of medium to large size, about 20 meters high, found in Mindoro, Mindanao, Basilan, and the Sulu Archipelago. The leaves are large, entire or lobed, dark green, and rough. The fruit is roundish, about the size of a child's head, thickly studded with upright blunt spines. The rind is thick and fleshy and contains a creamy white, sweet, juicy, and aromatic flesh of good taste. The large seeds may be roasted and eaten like peanuts. The maráng is one of the best "native" fruits of the Philippines.

CARISSA CARANDAS Linn. Plate 2, fig. 2.

A thorny shrub about 3 meters high or more, with long arching canes, native of India, from whence it was introduced several years ago. The white, fragrant, star-shaped flowers are followed by small clusters of ovoid to roundish black fruits about the size of a small cherry, with a subacid juicy pulp of pleasant taste. When green the fruit makes a good pickle, and when

[2] Wester, P. J., Bull. Philip. Bur. Agr. 39 (1925).

ripe it makes a fine jelly resembling grape jelly in color and taste.

The perunkila is very drought-resistant and is a promising fruit in regions with a long dry season. It makes an impenetrable live fence.

CUBILIA BLANCOI Blume. Kubili. Plate 3, fig. 1.

A tree of medium size of wide distribution but not common in the Philippines. The leaves are pinnate. The fruits are roundish oblong, bright green and spiny, 5 to 6 centimeters long and contain a roundish-oblong brown nut about 3 centimeters long. The fruit is of excellent quality, roasted or boiled, and the tree deserves to be generally planted.

EUGENIA POLYCEPHALOIDES C. B. Rob. Lipoti. Plate 3, fig. 2.

A small tree, 9 meters high or more, native of Albay, Samar, and probably several adjoining provinces. The young growth is quadrangular. The leaves are oblong-obovate and pointed, leathery, dark green, and shining. The flowers are small and whitish. The fruits are roundish, black and shining, with white, rather dry and crisp flesh of pleasant acid taste, borne in clusters upon tubercles from the stem, twenty to fifty or more fruits to a cluster. They make a good jelly.

GNETUM INDICUM (Lour.) Merr. Bulso. Plate 4, fig. 2.

A large woody vine of wide distribution at low and medium altitudes. The leaves are elliptic to ovate and pointed, about 15 centimeters long. The small flowers are followed by grape-like bunches of brick-red, one-seeded fruits about 25 millimeters long. The large starchy seed is eaten raw or roasted. This vine has been domesticated at Lamao.

LUCUMA NERVOSA A. DC. Canistel. Plate 4, fig. 1.

A small, handsome tree, about 7 to 9 meters high, native of the West Indies. The leaves are oblanceolate to oblong-obovate and bright green, about 15 centimeters long. The fruit is roundish to ovoid, frequently pointed, 5 to about 8 centimeters long, yellow, with yellow, somewhat mealy, sweet and aromatic flesh, inclosing one to three large seeds.

The canistel was introduced into the Philippines in 1912 and has proved successful both in moist and periodically dry regions.

MANGIFERA CAESIA Jack. Baúno. Plate 5, fig. 1.

A tree of medium to large size, 15 to 30 meters high, with straight trunk and of majestic habit. The leaves are long and pointed, leathery and prominently veined. The fruit is oblong-

obovoid, somewhat larger than a mango, pale green, thin-skinned, and with white, somewhat fibrous, very aromatic and subacid flesh in which a large seed is embedded. It is a native of Malaysia; and probably was introduced into the Philippines, where it was naturalized long ago.

MANGIFERA sp. Mango amini. Plate 5, fig. 2.[6]

Fruit small; 170 to 250 grams in weight; roundish oblong, oblique, flattened; base flat; dorsal shoulder short and rounded; apex rounded, with a prominent, pointed swelling at neck; surface smooth, bright yellow, with a heavy red or scarlet black coloring extending sometimes over more than one-half of the fruits well exposed to the light, lenticels numerous, light yellow; bloom heavy; skin thick, tough, and adhering to the flesh; this rich yellow in color, firm, juicy, fiberless, of pleasing flavor and rather strong aroma; seed large and thick, constituting a larger portion of the fruit than in most varieties.

The tree is of vigorous growth, tall and open, prolific, and a regular bearer; the fruiting season is unusually long.

The other variety of Indian mango (Plate 6, fig. 1) analyzed together with the amini has not been identified. However, it is very similar to the amini in size, color, and taste.

MOMORDICA COCHINCHINENSIS (Lour.) Spreng. Tabolo. Plate 6, fig. 2.

A robust vine often 15 meters long, of wide distribution at low and medium elevations. The leaves are broadly ovate, entire or 3-lobed and pointed, 10 to 18 centimeters long. The fruits are roundish to short-oblong, about 12 centimeters long, yellow, with scattered short soft spines. The fruits are eaten immature and boiled as a vegetable in the Ilocos provinces.

MUSA sp. Bananas. Plates 7, 8, and 9, fig. 1.

The bananas called ideep, gensombaba, and katali are very similar to gloria ternate in appearance, size, and taste; while the petri and the toybok resemble the inarnibal. The analyses of these bananas are given in Table 2.

NEPHELIUM MUTABILE Blume. Rulala. Plate 9, fig. 2.

A tree about 15 meters high, of wide distribution in humid regions at low elevations in the Philippines and Malaya. The leaves are pinnate. The fruits are borne in loose terminal clusters. They are short-oblong, greenish to reddish brown, with coarse, erect spines. In the cultivated varieties the whitish flesh is subacid, juicy, and of excellent taste; it contains a large seed.

[6] Wester, P. J., Bull. Philip. Bur. Agr. 33 (1922).

There is an excellent variety grown in Jolo, but the bulala is rarely planted elsewhere.

RESULTS

The results recorded in Tables 1 and 2 are interesting since these Philippine fruits are rather rare and not commonly found in Philippine markets. Considerable difficulty was experienced in securing characteristic samples of these fruits and in geting them to Manila in good condition for analysis.

Analyses given in the tables show a high water content, which is characteristic of fruits in general. The bulso (*Gnetum indicum*) and kubili (*Cubilia blancoi*) fruits gave a high protein content. The atemoya (*Anona* sp.) mangoes, and maráng (*Artocarpus odoratissima*) showed a comparatively high sugar content. Although the fat content of fruits is usually very low, the kubili (*Cubilia blancoi*) and perunkila (*Carissa carandas*) gave a remarkably high fat content (ether extract). The kubili also contains the highest ash, indicating a high mineral content.

SUMMARY

Eighteen fruits, including five kinds of bananas, were analyzed. As a means of identification, botanical descriptions and photographs of these fruits are recorded. The composition and the calorific value of these fruits were determined.

255381——12

TABLE 1.—*Composition of some Philippine fruits.*

Name of fruit.	Average weight.	Weight of edible portion.	Moisture.	Ash.	Protein.	Sugars.		Ether extract.	Crude fiber.	Acidity as acetic acid.	Other carbohydrates.	Calorific value per 100 grams.
						Sucrose.	Reducing.					
	g.	*g.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
Eugenia polycephaloides C. B. Rob.	5.1	.....	87.25	0.41	0.78	.....	2.77	1.51	1.56	2.13	3.59	49.71
Carissa carandas Linn.	4.9	.....	83.73	0.85	1.16	.....	9.65	2.24	1.15	.47	.75	72.94
Lucuma nervosa A. DC.	70.18	45.75	62.19	0.11	1.14	.....	9.10	1.18	1.29	.....	24.99	160.71
Nephelium mutabile Blume	29.30	6.0	84.54	0.45	0.82	.....	8.20	0.55	0.14	0.64	4.66	61.78
Cubilia blancoi Blume	13.5	.....	60.49	1.20	3.31	.....	.....	4.42	2.48	.....	28.10	180.08
Mangifera cuesia Jack	334.0	.....	82.73	0.46	1.59	.....	6.25	0.29	0.67	0.76	7.25	67.31
Artocarpus odoratissima Blanco	674.0	.....	84.23	0.51	1.47	4.37	6.17	0.23	0.77	0.13	2.07	63.02
Cretum indicum (Lour.) Merr.	4.5	.....	45.06	1.35	8.86	.....	.....	0.12	1.14	.....	48.37	220.34
Momordica cochinchinensis (Lour.) Spreng.	326.0	210	92.6	0.41	0.87	.....	Trace	0.48	0.94	.....	4.70	31.16
Artocarpus elastica Reinw.	260.00	.....	84.11	0.63	1.62	.....	7.24	0.37	0.92	0.30	4.81	63.26
Mango amini	195.0	72.0	79.72	0.42	0.83	11.21	5.90	0.25	0.61	0.96	.10	79.81
Mango, unidentified	189.5	68.0	79.74	0.50	0.33	7.42	6.45	0.31	0.48	0.30	4.47	81.40
Anona sp.	180.0	115.0	71.48	0.75	1.07	7.98	12.82	0.45	0.05	.....	5.40	116.20

TABLE 2.—*Composition of some Philippine bananas.*

Name of fruit.	Average weight.	Weight of edible portion.	Moisture.	Ash.	Protein.	Sugars.		Ether extract.	Crude fiber.	Acidity as acetic acid.	Other carbohydrates.	Calorific value per 100 grams.
						Sucrose.	Reducing.					
Bananas	*g.*	*g.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
Petri	37.0	32.0	73.82	0.65	1.02	6.73	15.87	0.43	0.80	.....	2.18	108.91
Toyhok	52.0	36.0	70.89	0.83	1.04	5.82	17.42	0.25	0.26	.....	3.99	117.23
Genesembaba	83.0	69.0	66.84	0.74	1.27	.....	20.53	0.53	0.41	.....	9.68	135.58
Katali	108.0	82.0	68.24	0.81	0.89	0.46	20.00	0.35	0.41	.....	8.84	128.72
Ideep	81.0	51.0	69.83	0.89	1.03	9.08	14.41	0.34	0.32	.....	4.10	121.82

ILLUSTRATIONS

PLATE 1

FIG. 1. *Anona* sp. Atemoya.
2. *Artocarpus elastica* Reinw. Gomihan.

PLATE 2

FIG. 1. *Artocarpus odoratissima* Blanco. Maráng.
2. *Carissa carandas* Linn.

PLATE 3

FIG. 1. *Cubilia blancoi* Blume. Kubili.
2. *Eugenia polycephaloides* C. B. Rob. Lipoti.

PLATE 4

FIG. 1. *Lucuma nervosa* A. DC. Canistel.
2. *Gnetum indicum* (Lour.) Merr. Bulso.

PLATE 5

FIG. 1. *Mangifera casia* Jack. Baúno.
2. *Mangifera* sp. Mango amini.

PLATE 6

FIG. 1. Mango (unidentified).
2. *Momordica cochinchinensis* (Lour.) Spreng. Tabolo.

PLATE 7

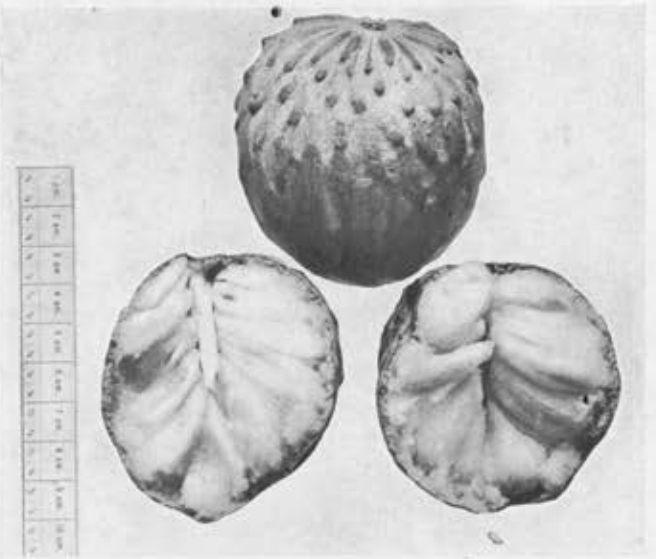
FIG. 1. *Musa* sp. Toybok.
2. *Musa* sp. Gensombaba.

PLATE 8

FIG. 1. *Musa* sp. Katali.
2. *Musa* sp. Ideep.

PLATE 9

FIG. 1. *Musa* sp. Petri.
2. *Nephelium mutabile* Blume. Bulala.



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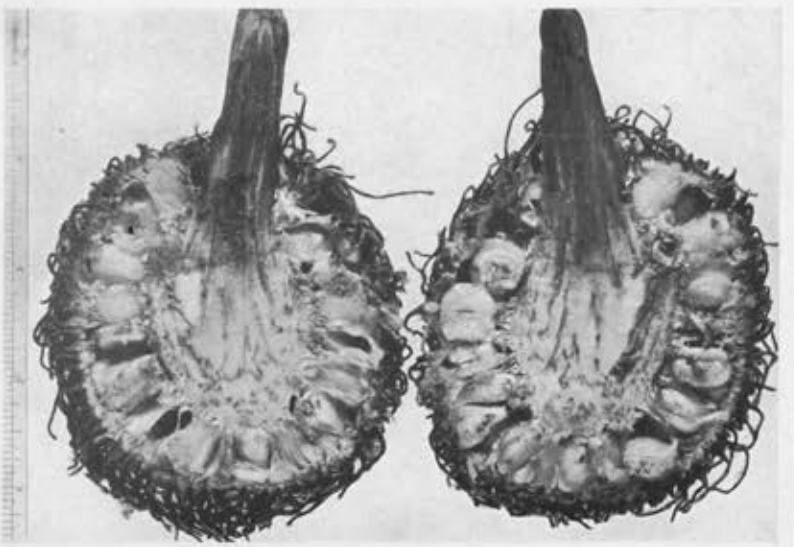

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Fig. 1. Anona sp.; 2. Artocarpus elastica.

PLATE 1.

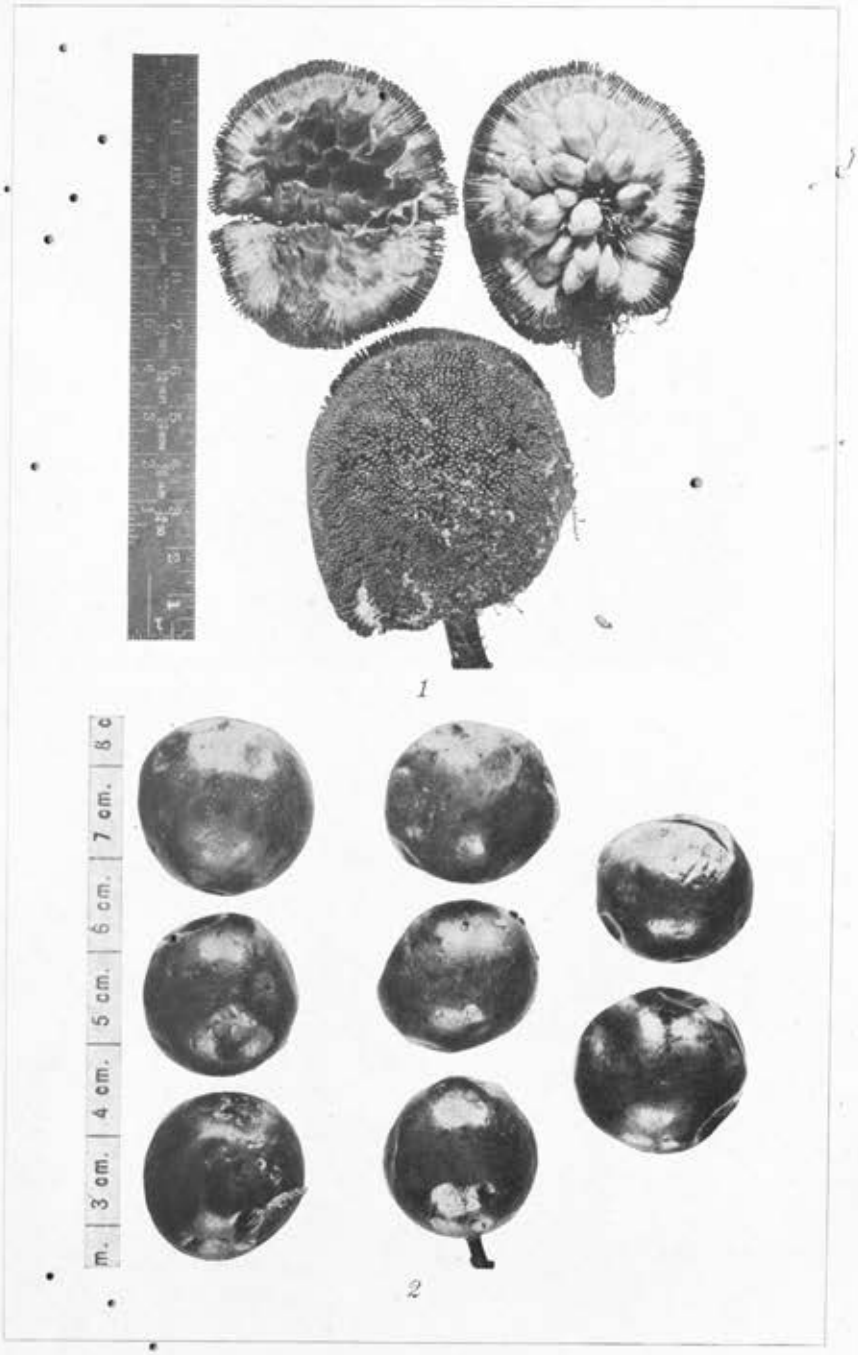


Fig. 1. Artocarpus odoratissima; 2. Carissa carandas.

PLATE 2.

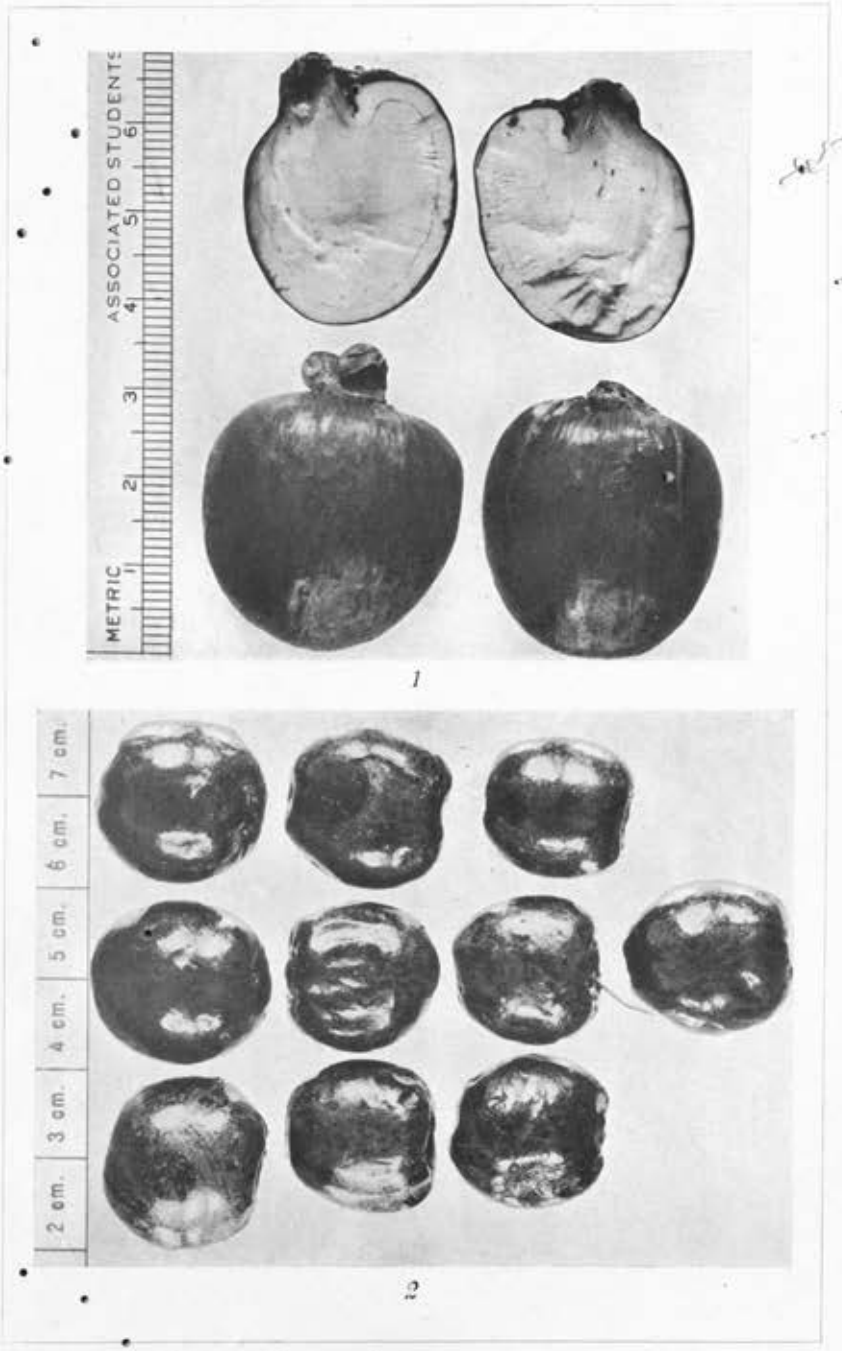


Fig. 1. Cubilia blancoi; 2. Eugenia polycephaloides.

PLATE 3.

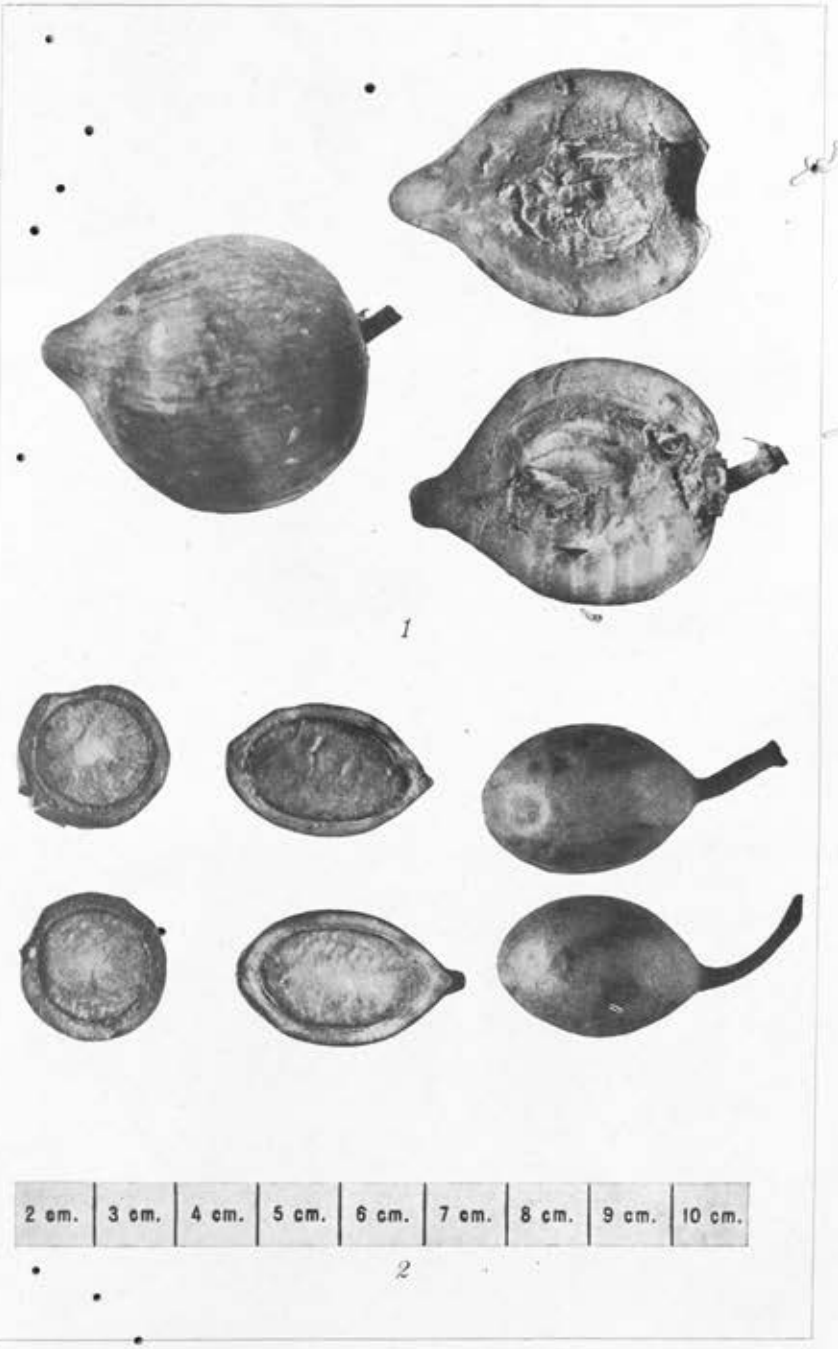


Fig. 1. Lucuma nervosa; 2. Gnetum indicum.

PLATE 4.

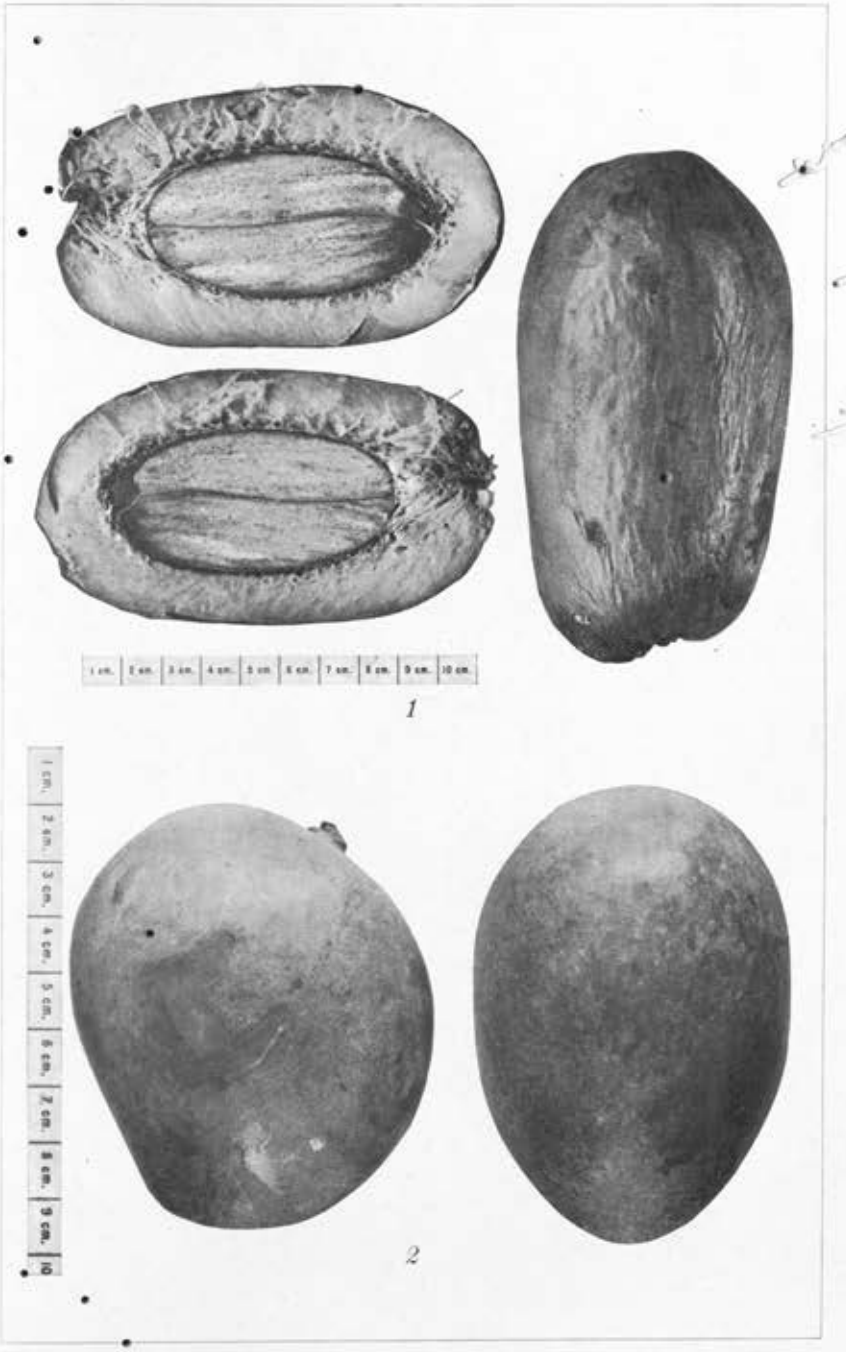
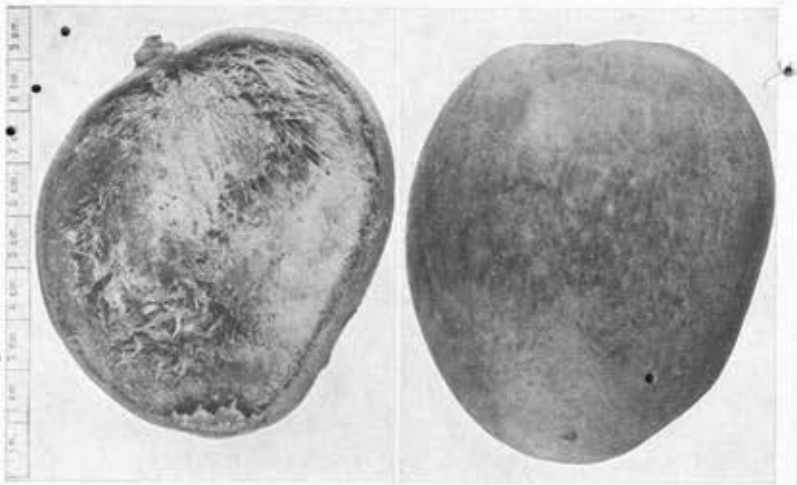


Fig. 1. Mangifera caesia; 2. Mangifera sp.

PLATE 5.



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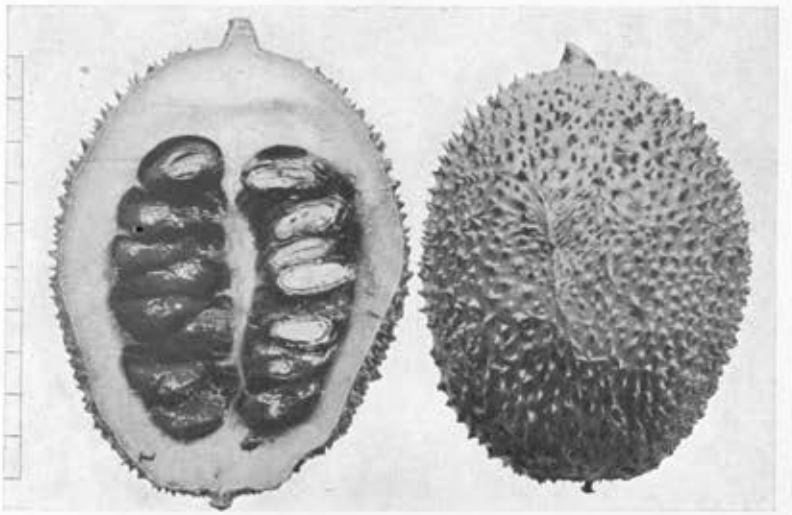

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Fig. 1. Mango (unidentified); 2. Momordica cochinchinensis.

PLATE 6.

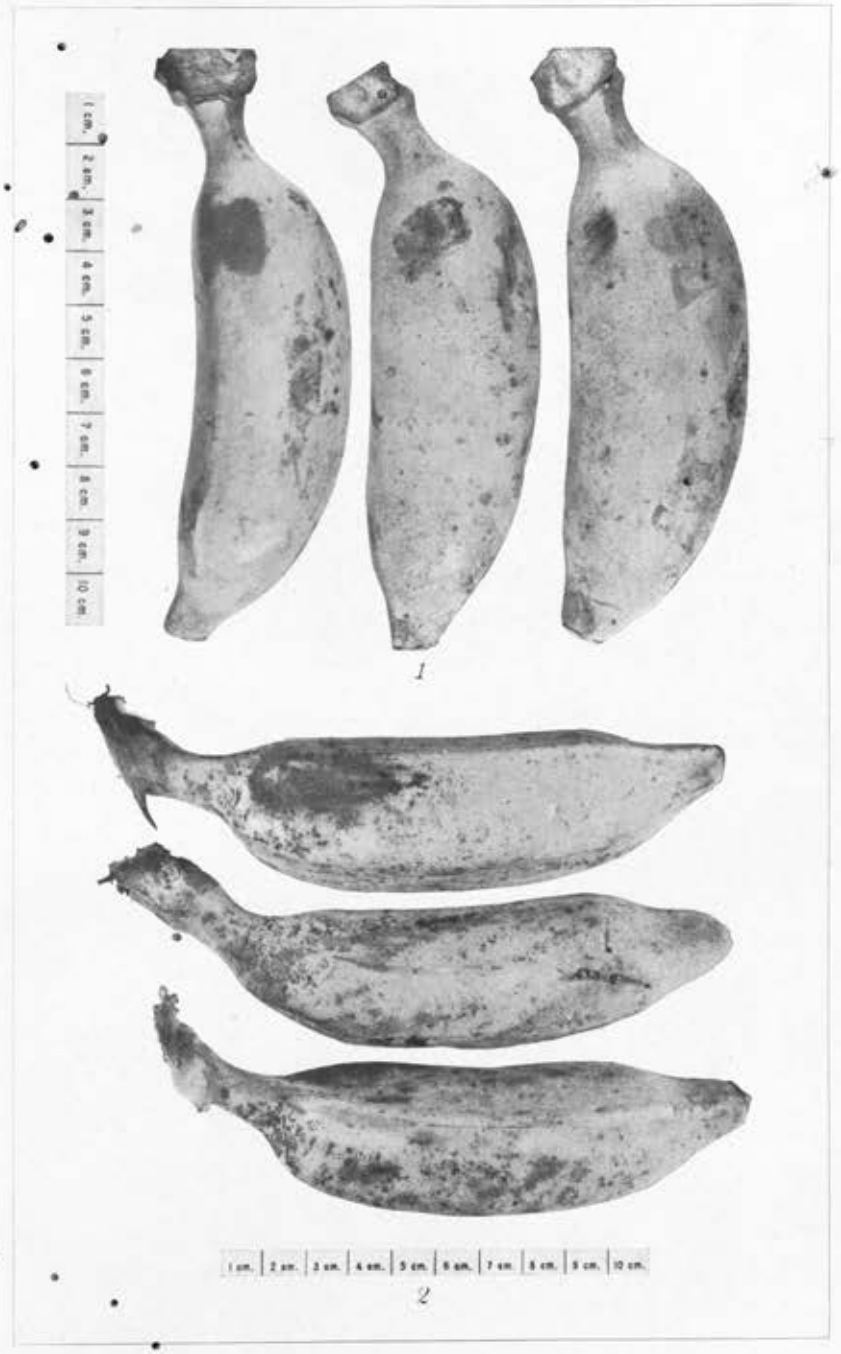


Fig. 1. Musa sp.; 2. Musa sp.

PLATE 7.

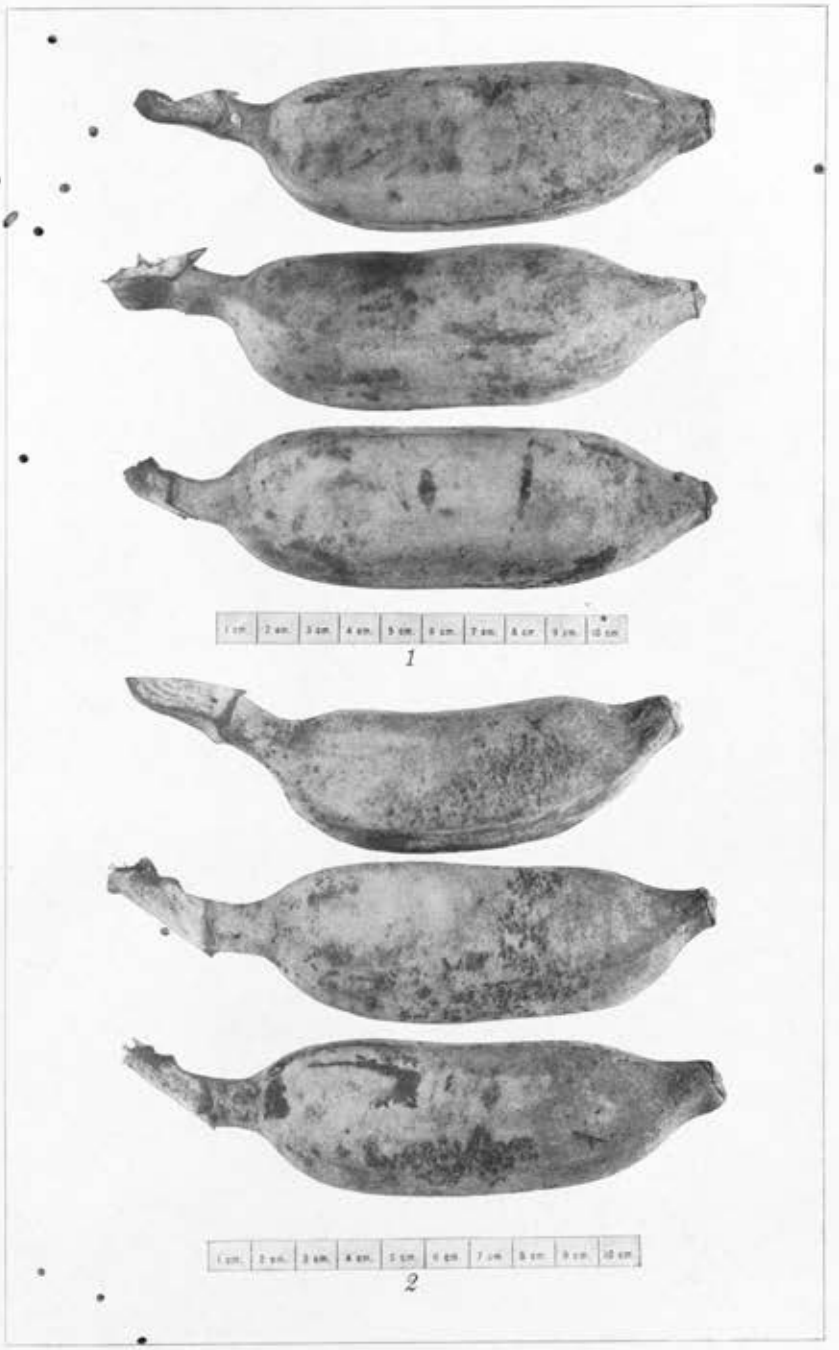
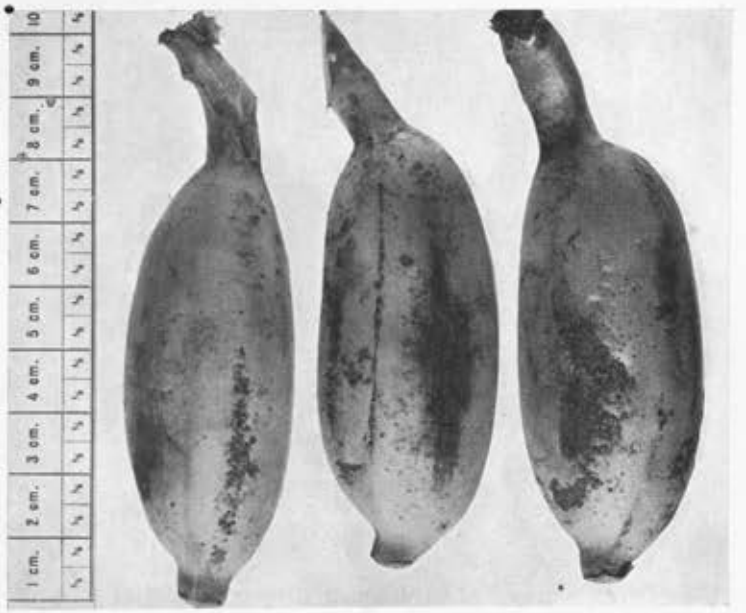


PLATE 8. MUSA SP.



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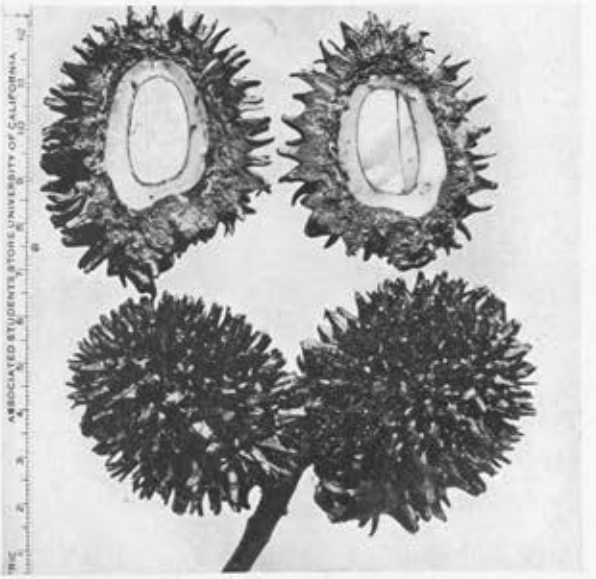

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Fig. 1. Musa sp.; 2. Nephelium mutabile.

PLATE 9.

INDEX

[New names and new combinations are printed in boldface.]

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